

Chapter 33

Lactic Acid Bacteria in Food Industry

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Abstract Lactic acid bacteria (LAB) are known through ages for their wide applications in food, pharmaceutical and chemical industries. But recently LAB have aroused interest for their ability to secrete extracellular polysaccharides or glucans. These glucans have immense commercial value because of their industrially useful physico-chemical properties. The glucans derived from LAB play crucial role in improving rheology, texture, mouth feel of fermented food formulations and conferring beneficial physiological effects on human health, such as antitumour activity, immunomodulating bioactivity and anticarcinogenicity. The modulation of biochemical properties of glucans require a thorough understanding of its biosynthetic pathway and the relation between the structure of glucans and the functional effect provided by them after incorporation into the food matrix. LAB are employed in food industry for making yoghurt, cheese, sourdough bread, sauerkraut, pickles, beer, wine and other fermented foods and animal feeds like silage. LAB can also produce a variety of functional oligosaccharides that have applications as prebiotics, nutraceuticals, sweeteners, humectants, drug against colon cancer and as immune stimulator. LAB are gram positive rods or cocci, non spore forming, acid tolerant, low GC containing, anaerobic or micro-aerophilic bacteria characterized by their ability to ferment sugar to lactic acid. The commonly known LAB genera are *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Lactococcus* and *Streptococcus*. Besides prolonging the shelf life, lactic acid enhances the gustatory and nutritional value, imparts appetizing flavour and texture to the food. Some LAB produce proteinaceous antimicrobial compounds called bacteriocins which inhibit the growth of Gram-positive pathogenic and spoilage bacteria and used as food additives. Lactic acid bacteria as probiotics have been proven effective against diarrhoea, irritable bowel disorder, allergies, stimulation of immunity, lactose intolerance.

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33.1 Introduction

Lactic acid bacteria are industrially important and beneficial microbes that have similar properties (gram positive and catalase negative) and all produce lactic acid as an end product of the fermentation process. The genera *Carnobacterium*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Lactosphaera*, *Leuconostoc*, *Melissococcus*, *Oenococcus*, *Pediococcus*, *Streptococcus*, *Tetragenococcus*, *Vagococcus* and *Weissella* are recognized as LAB (Ercolini et al. 2001; Holzapfel et al. 2001). They are widespread in nature and are also found in our digestive systems. There are several potential health or nutritional benefits possible from several species of lactic acid bacteria, among these are: improved nutritional value of food, control of intestinal infections, improved digestion of lactose, control of some type of cancer and control of serum cholesterol level. Although they are best known for their role in the preparation of fermented dairy products, they are also used for pickling of vegetables, baking, wine-making, curing fish, meats and sausages. *Lactobacillus plantarum* and *Lactobacillus sanfrancisco* are commercially available and widely used e.g. for the production of fermented milk products and for the preparation of sourdough (Sing 1977). LABs are also regarded as a major group of probiotic bacteria (Schrezenmeir and de Vrese 2001) i.e. they are a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance. LAB produces various types of exopolysaccharide which has numerous application in food and pharmaceutical industry (Naessens et al. 2005; Purama and Goyal 2005) with the help of extracellular glucansucrase enzyme. These glucansucrase produce three types of glucans based on the types of the linkages present. They are dextran, mutan and alternan. The dextran is composed of mainly $\alpha(1 \rightarrow 6)$ linkage and occasionally $\alpha(1 \rightarrow 2)$, $\alpha(1 \rightarrow 3)$ and $\alpha(1 \rightarrow 4)$ linkage as branching points (Monchois et al. 1999). Mutan is a type of insoluble glucan having more than 50% $\alpha(1 \rightarrow 3)$ linkages (Mooser 1992) and alternan composed of alternating $\alpha(1 \rightarrow 6)$ and $\alpha(1 \rightarrow 3)$ glucosidic linkages, with some degree of $\alpha(1 \rightarrow 3)$ branching (Seymour and Knapp 1980; Cote and Robyt 1982). LAB can also produce a variety of functional oligosaccharides synthesized by glucansucrase as a result of acceptor reaction (Demuth et al. 2002) and can be used as used as neutraceuticals, stabilizers and prebiotics (Goulas et al. 2004; Naessens et al. 2005).

33.2 LAB in Food Industry

Lactic acid bacteria is used throughout the world to produce specialty foods, particularly in fermented milk products, including yoghurt, cheese, butter, buttermilk, kefir. Some of the traditional fermented food products and their associated

Table 33.1 Fermented food products and associated bacteria

Type of fermented food	Lactic acid bacteria	Country	Sources
Kimchi	<i>Leuconostoc mesenteroides</i> , <i>Lactobacillus curvatus</i> , <i>Lactobacillus brevis</i> , <i>Lactobacillus sake</i> and <i>Lactobacillus plantarum</i>	Korea	Fermented mixture of Chinese cabbage, radishes, red pepper, garlic and ginger
Kefir	<i>Lactobacillus kefir</i> , <i>Lactobacillus brevis</i>	Russia	Fermented milk product
Swiss cheese	<i>Lactobacillus lactis</i> , <i>Lactobacillus delbrueckii</i>	Switzerland	Dairy product
nam	<i>Weissella cibaria</i> , <i>Leuconostoc citreum</i>	Thailand	Fermented fresh Pork
Magou	<i>Leuconostoc mesenteroides</i> , <i>Pediococcus cerevisiae</i> , <i>Streptococcus lactis</i>	South africa	Fermented maize porridge
Balao balao	<i>Streptococcus sp.</i> , <i>Leuconostoc sp.</i> , <i>Pediococcus sp.</i>	Phillipines	Fermented rice and shrimp mixture
Gari	<i>Lactobacillus pentosus</i> , <i>Leuconostoc fallax</i> , <i>Weissella paramesenteroides</i> <i>Lactobacillus fermentum</i>	Nigeria	Fermented cassava
Kishk	<i>Lactobacillus sakei</i> , <i>leuconostoc sp.</i>	Egypt	Fermented cereal and milk mixture
Laban rayeb	<i>Streptococcus faecalis</i>	Egypt	Fermented milks
Ras cheese	<i>Lactococcus sp.</i> , <i>Lactobacillus sp.</i> , <i>Enterococcus sp.</i> , and <i>Pediococcus sp.</i>	Egypt	Dairy product
Sauerkraut	<i>Leuconostoc mesenteroides</i> , <i>Lactobacillus plantarum</i> , <i>Pediococcus acidilactici</i>	Western countries	Fermented cabbage
Sourdourgh	<i>Lactobacillus fermentum</i> , <i>Lactobacillus brevis</i> , <i>Lactobacillus plantarum</i> , <i>Lactobacillus panis</i> , <i>Weissella cibaria</i>	Europe	Fermented cereals

lactic acid bacteria are listed in Table 33.1. LAB are either homofermentative or heterofermentative based on the organism's metabolic pathway. Homofermentative bacteria such as *Lactococcus* and *Streptococcus* yield two lactates from one glucose molecule, whereas the heterofermentative bacteria such as *Leuconostoc* and *Weissella* transform a glucose molecule into lactate, ethanol and carbon dioxide (Kuipers et al. 2000). Lactic acid bacterial also produce acetic acid, aroma compounds, bacteriocins and exopolysaccharide and several important enzyme. For example acetaldehyde, provides the characteristic aroma of yoghurt, while diacetyl imparts a buttery taste to other fermented milks which improve the taste quality because at the fermentation process by lactic acid bacteria produce lactic

Table 33.2 Type of dextran produced by *Leuconostoc* strain and their source (Purama and Goyal 2005)

Strain	Type of dextran	Source
<i>Leuconostoc mesenteriodes</i> NRRL B-512F	1,3-branched dextran	Root beer
<i>Leuconostoc dextranicum</i> NRRL B-1121	1,3-branched dextran	
<i>Leuconostoc mesenteriodes</i> NRRL B-523	1,3-branched dextran w/1,3-linear segments	
<i>Leuconostoc mesenteriodes</i> NRRL B-1118	1,3-branched dextran w/1,3-linear segments	Olives
<i>Leuconostoc mesenteriodes</i> NRRL B-1298	1,2-branched dextran	Cane juice
<i>Leuconostoc mesenteriodes</i> NRRL B-1299	1,2-branched dextran	
<i>Leuconostoc mesenteriodes</i> NRRL B-1397	1,2-branched dextran	Sugar cane
<i>Leuconostoc mesenteriodes</i> NRRL B-1399	1,2-branched dextrans	Cane stubble
<i>Leuconostoc mesenteriodes</i> NRRL B-1422	1,2-branched dextran	Orange concentrate
<i>Leuconostoc mesenteriodes</i> NRRL B-1424	1,2-branched dextran	Refined sugar
<i>Leuconostoc mesenteriodes</i> NRRL B-1297	1,2- or 1,4-branched dextran	
<i>Leuconostoc mesenteriodes</i> NRRL B-1402	1,2- or 1,4-branched dextran	Orange concentrate
<i>Leuconostoc mesenteriodes</i> NRRL B-1431	1,2- or 1,4-branched dextran	Cane juice
<i>Leuconostoc mesenteriodes</i> NRRL B-1433	1,2- or 1,4-branched dextran	Cane juice
<i>Leuconostoc mesenteriodes</i> NRRL B-1149	1,3 and 1,2- or 1,4-branched dextran	
<i>Leuconostoc dextranicum</i> NRRL B-1420	1,4-branched dextran	Mill slime
<i>Leuconostoc citreum</i> NRRL B-742	1,3-branched + 1,4-branched dextran fractions	
<i>Streptobacterium dextranicum</i> NRRL B-1254	1,3-branched + 1,4-branched dextran fractions	
<i>Leuconostoc mesenteriodes</i> NRRL B-1375	1,3-branched + 1,2- or 1,4-branched dextran fractions	
<i>Leuconostoc mesenteriodes</i> NRRL B-1377	1,3-branched + 1,2- or 1,4-branched dextran fraction	
<i>Leuconostoc mesenteriodes</i> NRRL B-1374	1,3-branched dextran	
<i>Leuconostoc mesenteriodes</i> NRRL B-1355	Alternan + Dextran	
<i>Leuconostoc mesenteriodes</i> NRRL B/11011		

(continued)

Table 33.2 (continued)

Strain	Type of dextran	Source
<i>Leuconostoc mesenteroides</i> PCSIR-3		Cabbage and Carrot
<i>Leuconostoc mesenteroides</i> NRRL B-1501		
<i>Leuconostoc mesenteroides</i> IBT-PQ		'Pulque' (alcoholic beverage)
<i>Leuconostoc dextranicum</i> FPW-10		Fermenting palm wine
<i>Leuconostoc mesenteroides</i> L. <i>Leuconostoc mesenteroides</i> Lcc4 <i>Leuconostoc dextranicum</i> strain elai		

acid in fermented milks which gives slightly tart taste. Some of the fermented foods produced by lactic acid bacteria are listed in Table 33.2 (Avonts et al. 2004).

33.3 Lactic Acid in Food Industry

Lactic acid is water soluble, non-volatile odorless and is classified as GRAS (generally regarded as safe) by FDA in the USA. Several lactic acid bacteria produce lactic acid at the end of carbohydrate fermentation. The presence of lactic acid, produced during the lactic acid fermentation is responsible for the sour taste and for the improved microbiological stability and safety of the food. Lactic acid is produced in the form of L(+) or D(-) lactic acid or as its racemic mixture. Organisms that form the L(+) form or D(-) form have two lactate dehydrogenases (LDH), which differ in their stereospecificity. Some *Lactobacilli* produce L(+) form, which on accumulation induces a racemase, which converts it into D(-) lactic acid until equilibrium is obtained. *Lactobacillus plantarum* produce both L(+) form or D(-) form. Of these L(+) form is of great application for its ability to tolerate high concentration of hydrogen ions and pure form of L(+) lactic acid is produced from *Lactobacillus helveticus* by metabolic engineering (Nikkila et al. 2000).

Lactic acid is used as acidulant, flavorings and pH buffering agent or inhibitor of bacterial spoilage in a wide variety of processed foods. In contrast to other food acids it has a mild acidic taste. It is a very good preservative and pickling agent. Addition of lactic acid aqueous solution to the packaging of poultry and fish increases their shelf life. The esters of lactic acid are used as emulsifying agents in baking foods (stearoyl-2-lactylate, glyceryl lactostearate, glyceryl lactopalmitate). The manufacture of these emulsifiers requires heat stable lactic acid, hence only the synthetic or the heat stable fermentation grades can be used for this application (Sodegard 1998).

33.4 Glucans from LAB in Food Industry

Lactic acid bacteria produce a wide variety of food grade exopolysaccharides (EPS) with the help of glucosyltransferases that have nutritional and health applications. Glucansucrases are large extracellular enzymes capable of synthesizing various glucans from sucrose, such as dextran, mutan and alternan. These exopolysaccharides are potential therapeutic agents (Korakli and Vogel 2006) and are also used as viscosifying, stabilizing, emulsifying, sweetening, gelling or water-binding agents, in the food as well as in the non-food industries (Sutherland 1998; Welman and Maddox 2003). β -glucan consist of linear unbranched polysaccharides of linked β -(1 \rightarrow 3) and β -(1 \rightarrow 4)-D-glucopyranose units, and it is a natural water-soluble fiber that cannot be digested by human enzymes, but is degraded by bacteria in the colon into short-chain fatty acids (SCFAs). LAB strains belonging to the genera *Pediococcus*, *Lactobacillus* and *Oenococcus* isolated from cider and wine, produce a 2-substituted (1 \rightarrow 3)-D-glucan (Dols-Lafargue et al. 2008). The plasmid *gtf* gene determinant for EPS production was cloned into *Escherichia coli* and determination of its DNA sequence revealed that it encodes a protein, named GTF glycosyltransferase. Cloning of the *gtf* gene and functional expression of its encoded glycosyltransferase in *Streptococcus pneumoniae* (Werning et al. 2006) and *Lactococcus lactis* revealed that this enzyme is indeed responsible for the synthesis of the β -D-glucan (Werning et al. 2008).

Glucan has several importances in food industry for its soluble nature which form viscous aqueous solutions and increases the viscosity of gut contents in the human alimentary tract. Now soluble fibers are very much concerned in human nutrition (Dols-Lafargue et al. 2008).

1. *Cholesterol lowering effect*: it absorbed the cholesterol, helps in lowering the blood cholesterol level, thus reduce the risk of cardiovascular disease.
2. *Lowering effect of the glycaemic index*: Glucans are very viscous in nature which makes the gastric content thicker and help in slowing down the absorption rate of glucose. β -glucan thus spread glucose absorption over time (i.e. *reduction of glycaemic index*) and helps the body to fight against diabetes. In food industry, beside classical applications of polysaccharides as thickening agent, β -glucans have an increasing interest in the areas of edible film and as stabilizers in the manufacture of low-fat products such as salad dressings (Kontogiorgos et al. 2004), ice creams and yoghurts (Brennan et al. 2002) and cheese.

Four different genera of lactic acid bacteria, *Streptococcus*, *Leuconostoc*, *Pediococcus* and *Lactobacillus* are known to produce glucan. Glucan synthesis in lactic acid bacteria has been mainly studied from *Leuconostoc* spp. (Monsan et al. 2001; Majumder et al. 2009; Purama et al. 2009) with the help of glucansucrase enzyme. The culture condition of novel glucan and glucan producing glucansucrase from *Leuconostoc* spp. has been optimized by statistical approach method. Various types of glucan produced by *Leuconostoc* species and their source are given in Table 33.2. Glucansucrase (or also called dextransucrase) (E.C. 2.4.1.5) catalyzes

the polymerization of the glucopyranosyl moieties of sucrose to form dextran (Purama and Goyal 2008). Dextran are used as viscosifying, texturizing or gelling agents in food formulations (Majumder and Goyal 2009), due to its non-ionic character and good stability under normal operating condition.

L. mesenteroides NRRL B-640 is shown to produce dextransucrase that gives highly linear and soluble dextran (Uzochukwu et al. 2002). A novel dextran produced by *Leuconostoc dextranicum* NRRL-B-18242 having a slushy, applesauce-like appearance with a particulate gel-like structure was described and the dextran in particulate or dried form is used in foods and other applications where texture is important (Pucci and Kunka 1990). *Leuconostoc mesenteroides* NRRLB-512 F is also used industrially to produce dextransucrase, which synthesize extracellular homopolysaccharide dextran (Purama and Goyal 2005).

The dextran is commercialized in a powder form and as a liquid with the following specifications

1. For the powdered form (values in % of commercial product) carbohydrate 60 (with dextran 50, mannitol 0.5, fructose 0.3, leucrose 9.2), protein 6.5; lipid 0.5; lactic acid 10; ethanol traces; ash 13; moisture 10.
2. For the liquid form (values in % of commercial product): carbohydrate 12 (with dextran 6.9; mannitol 1.1; fructose 1.85; leucrose 2.15); protein 2; lipid 0.1; lactic acid 2; ethanol 0.5; ash 3.4; moisture 80.

The solubility of dextran depends upon the branched linkage pattern. Presence of 95% linear linkages makes this dextran water-soluble, which makes it suitable for various applications (Leathers 2002).

The micro-organisms used for the production of dextran (*Leuconostoc mesenteroides*, *Lactobacillus plantarum*, *Lactobacillus sanfrancisco*) are currently used in food processing without any restriction. As a result of microbial activity, dextran occurs in small amounts in naturally fermented products such as sauerkraut and cucumber and in kefir where it probably plays a role in the thickening (Roller and Dea 1992). It has been reported that the glucan produced by *Leuconostoc dextranicum* NRRL B-1146, having $\alpha(1 \rightarrow 4)$ and $\alpha(1 \rightarrow 6)$ linkages, showed non-Newtonian pseudoplastic behaviour (Fig. 33.1) indicating its branched nature and also have unique rheological properties because of its potential of forming very viscous solution at low concentration and can be used as thickening or gelling agent in food.

Further the Scanning Electron Micrograph (shown in Fig. 33.2) of the glucan produced by *Leuconostoc dextranicum* NRRL B-1146 showed small porous or web like structure, that facilitates its water holding capacity, thus can be used as a texturing agent in food industry (Majumder and Goyal 2009). The surface morphology of dried and powdered dextran from *Leuconostoc dextranicum* NRRL B-640 was also studied using Scanning Electron Microscopy and it revealed the cubical porous structure of dextran, as shown in Fig. 33.3. It was recently reported that the dextran from *Pediococcus pentosaceus* holds potential usage as gelling agent in food formulations and as drug delivery carriers (Patel et al. 2010).

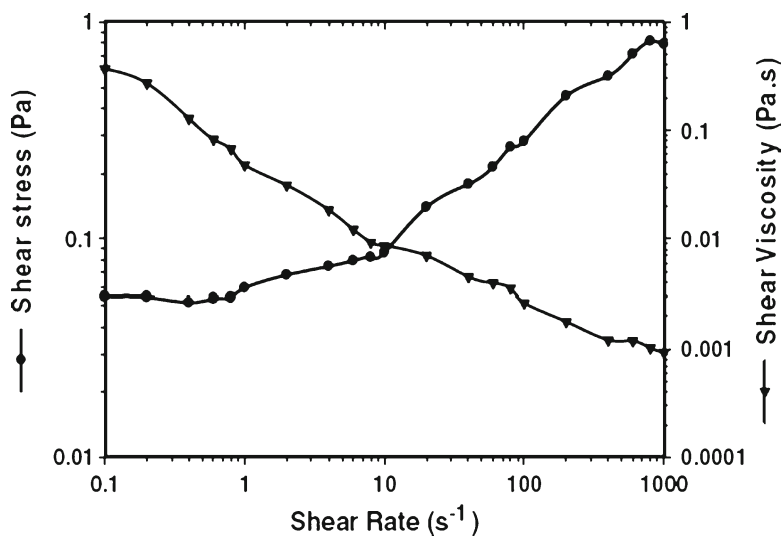


Fig. 33.1 The steady shear measurements for the semi-dilute glucan solution from *Leuconostoc dextranicum* NRRL B-1146 indicated that the viscosity (η) of the dilute polymer exponentially decreased with the increase in shear stress (τ) and exhibited a typical non-Newtonian pseudoplastic behavior (Majumder and Goyal 2009)

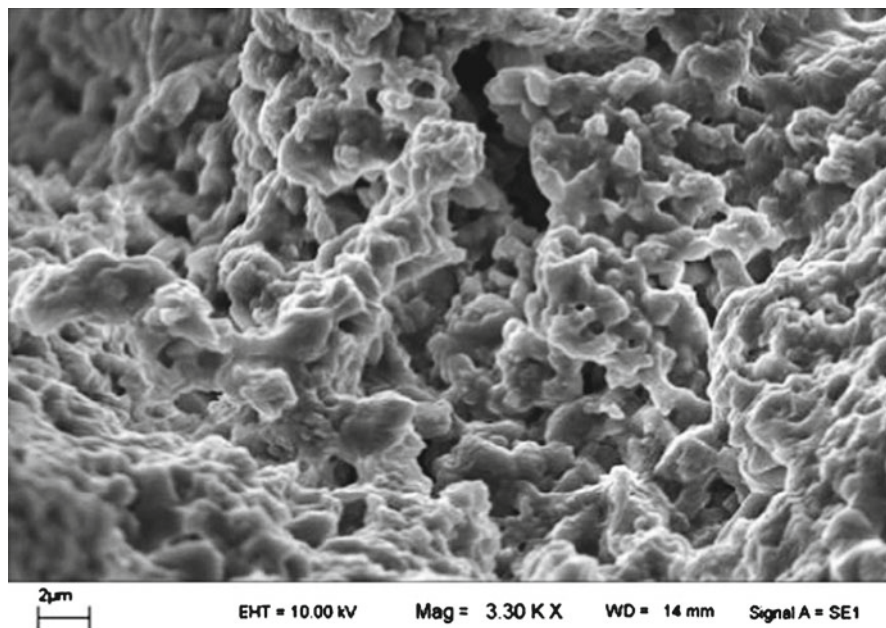


Fig. 33.2 Scanning Electron Micrograph of glucan showing surface morphology of *Leuconostoc dextranicum* NRRL B-1146 (Majumder and Goyal 2009)

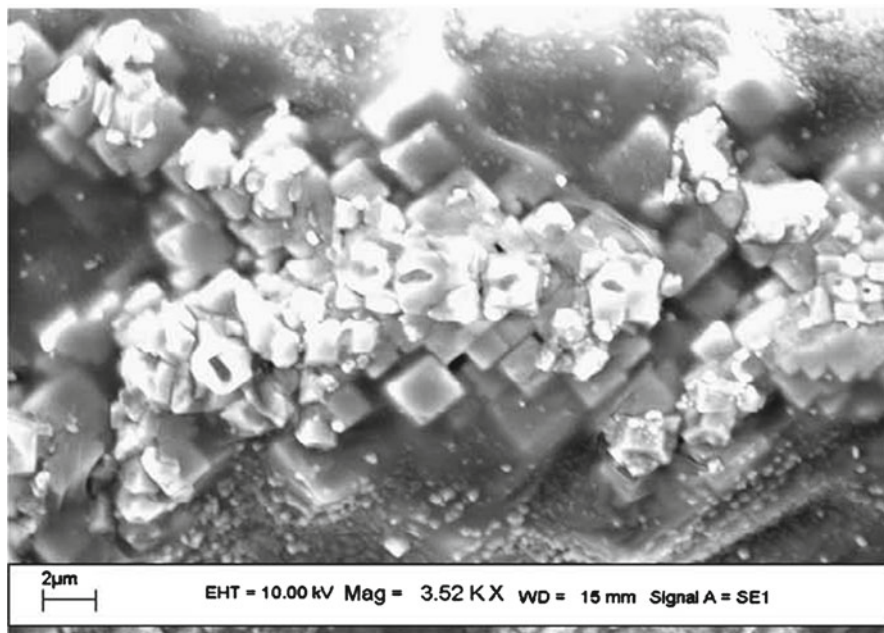


Fig. 33.3 Scanning Electron Micrograph of glucan showing surface morphology from *Leuconostoc dextranicum* NRRL B-640 (Purama et al. 2009)

The cytotoxicity test of dextran from *Pediococcus pentosaceus* was explored using human cervical cancer (HeLa) cell line and it is reported that there is no effect of dextran from *P. pentosaceus* on the viability of HeLa cells when observed for 72 h even at high concentration of 1,000 $\mu\text{g/ml}$ (Fig. 33.4) (Patel et al. 2010). This revealed that the dextran is non-toxic and biocompatible, rendering it safe for drug delivery, tissue engineering and various other biomedical applications.

33.5 Oligosaccharides from LAB in Food Industry

Certain oligosaccharides (e.g. fructooligosaccharides, isomaltooligosaccharides and lactulose) and polysaccharides (e.g. fructans) are used as prebiotic foods (Monsan and Paul 1995). These oligosaccharides contains high proportion of $\alpha(1 \rightarrow 6)$ glycosidic linkages that are partly or totally resistant to attack by human's and animal's digestive enzymes. Prebiotic oligosaccharides are noncarcinogenic, nondigestible and low calorific compounds stimulating the growth and development of gastrointestinal microflora described as probiotic bacteria such as *Bifidobacteria*

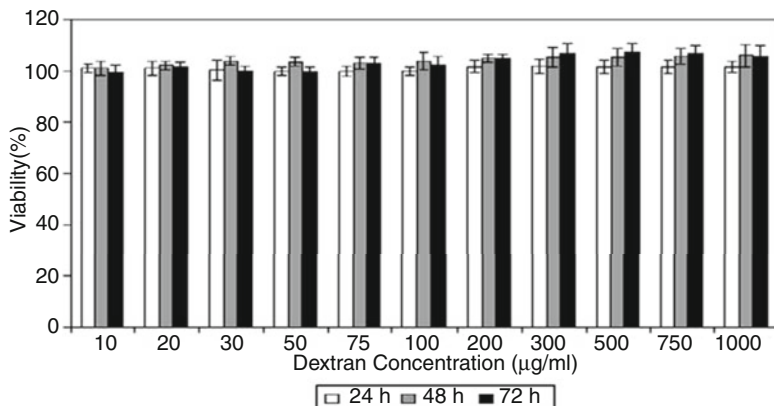


Fig. 33.4 The indirect contact based in vitro cytotoxicity assay showing the cell viability was unaltered after treatment with various concentrations of dextran (10–1,000 µg/ml) from *Pediococcus pentosaceus* over a period of 24–72 h incubation (Patel et al. 2010)

and *Lactobacilli* (Kubik et al. 2004). They are not absorbed in small intestine and in the large intestine they are metabolized by the colonic bacterial flora and fermented into short chain fatty acids. The effects of prebiotics are principally due to stimulation of the growth of bifidobacteria (bifidogenic effect). The stimulation of this growth allows a reduction in the pH of the colon, an increase in the production of short chain fatty acids (SCFAs), in particular butyrate and propionate, a prevention of the installation of pathogenic microorganisms (barrier effect), an increase in the metabolization of potentially carcinogenic aminated compounds and the production of vitamin B.

Gluco-oligosaccharides have been produced by enzymatic synthesis using glucosyl donor and an acceptor molecule (Iliev et al. 2008). Gluco-oligosaccharides production from glucan by microwave assisted hydrolysis from *Leuconostoc mesenteroides* NRRL B-742 has also been reported. The peak at ~527 m/z value using MALDI-TOF-MS showed the presence of three glucose units of (m/z ~180) and degree of polymerization of 3 (DP-3) for the oligosaccharide that was obtained from the glucan sample hydrolysed for 2 min.

The oligosaccharides are used widely in various food products such as soft drinks, cookies, cereals, candies and dairy products (Barreteau et al. 2006). For instance, galactooligosaccharides have shown very promising results increasing populations of both *Lactobacilli* and *Bifidobacteria* and increasing beneficial short chain fatty acids (Smiricky-Tjardes et al. 2003). A mixture of galactooligosaccharides and fructooligosaccharides added to standard infant formulas has also been shown to increase both *Lactobacilli* and *Bifidobacteria* species in human infants (Moro et al. 2002). Other applications for oligosaccharides such as an anticarcinogenic agent or a low-sweetness humectant have been explored (Chung and Day 2002).

33.6 Bacteriocin from LAB in Food Industry

A large number of ribosomally synthesized bacteriocins or bacteriocin-like substances are produced by lactic acid bacteria (LAB) have been identified and characterized in recent years due to their antimicrobial activity against foodborne pathogenic, as well as spoilage bacteria. The antibacterial spectrum frequently includes spoilage organisms and food-borne pathogens such as *Listeria monocytogenes* and *Staphylococcus aureus*. For these it has raised considerable interest for their application in food preservation that help to reduce the addition of chemical preservatives and/or the intensity of heat and other physical treatments, thus satisfying the demands of consumers for foods that are fresh tasting, ready to eat and lightly preserved. Bacteriocin can be added to foods in the form of concentrated preparations as food preservatives, shelf-life extenders, additives and ingredients (*ex-situ*) or they can be produced in situ by bacteriogenic starters, adjunct or protective cultures (Galvez et al. 2007). *In situ* bacteriocin production offers several advantages compared to *ex situ* production regarding both legal aspects and costs. Bacteriocins are usually inactivated by low pH, heat and from digestive enzyme such as proteases. Nisin, the product of some strains of *Lactococcus lactis* subsp. *lactis*, which was accorded GRAS (generally recognized as safe) status and approved for food use by the U.S. Food and Drug Administration, has already found a variety of applications in food preservation (Twomey et al. 2002). Till date only nisin and pediocins have been used as biopreservatives in food systems (Ray 1992; Rodríguez et al. 2002). Up to now, bacteriocins have been isolated from the commercial probiotic strains *Lactobacillus casei* Shirota and *Lactobacillus johnsonii* La1 (Avonts et al. 2004). Immobilized bacteriocins can also find application for development of bioactive food packaging. The effectiveness of bacteriocins requires careful testing in the food systems for which they are intended to be applied against the selected target bacteria.

33.7 LAB as Probiotic

Lactic acid bacteria mainly *Lactobacilli*, *Streptococci*, *Enterococci* and *Lactococci* can be used as probiotic (Schrezenmeir and de Vrese 2001). They possess the property by which they can be considered as probiotic such as colonization or adhesion properties, good *in vitro* development, ability of cells to produce metabolites and enzymes, stability in bile and gastric juices, production of antimicrobial substances, antagonistic action against noted pathogenic bacteria and/or viruses and no adverse interactions with host especially, in terms of pathogenicity. LAB also potentially used as starter cultures for the manufacture of dairy-based probiotic foods (Saarela et al. 2002). Probiotic acidophilus is a naturally occurring antibiotic that helps enhance digestion produces vitamin B and brings down the risk of colon cancer. In addition, antimicrobial production by probiotic LAB might play a role during *in vivo* interactions occurring in the human gastrointestinal tract, hence contributing to gut

Table 33.3 Probiotic food and their applications

Food	Microrganism	Manufactured By	Uses
Actimel	<i>L.bulgaricus</i> , <i>S. thermophilus</i> and <i>L. casei</i> .	Dannon Company	Used as delicious sweet milk
Align	<i>Bifidobacterium infantis</i> 35624		Found in clinical studies to help build and maintain a healthy digestive system as well as benefitting those suffering from irritable bowel syndrome (IBS)
LC1	<i>Lactobacillus johnsonii</i>	Nestle	Used as baby food to reduce the risk of infant diarrhea
Lifeway Kefir	<i>L. lactis</i> , <i>L. cremoris</i> , <i>L. diacetylactis</i> , <i>L. casei</i>	Lifeway and is available at Wild oat markets	Used in many food products in order to improve immune system

health. Probiotics may regulate local and systemic immunity, thereby reducing allergic disease severity and susceptibilities of infants and children to allergies and atopic diseases (Hsieh and Versalovic 2008). There are several examples of probiotic food that is commercially available shown in Table 33.3.

33.8 Polyols Produced by LAB

Several LAB produce polyols which are most commonly used as sugar replacement agents in food industry. Polyols are known as sugar alcohols and although they have the structure of alcohol, they mimic the sweetness of sugar when added to food. Sugar alcohols have a lower calorie count than true sugar because they are not entirely absorbed by the body and are often used in sugar-free or diet foods and drinks. One product that almost invariably contains sugar alcohols is chewing gum. They are added to gum because they are not broken down by either saliva or chewing action and therefore do not cause tooth decay. Some of the most common sugar alcohols are maltitol, sorbitol which are produced by LAB. *Leuconostoc fructosum* NRRL B-2041 produced manitol with maximum volumetric productivity of 2.36 g/l h when grown in supplemented carob syrup medium (Carvalho et al. 2010). All of these sugar alcohols produced by LAB have different ratings of sweetness. Sorbitol is 60% as sweet as true glucose, while maltitol rates at 75%. Sorbitol is synthesised by an engineered *Lactobacillus casei* and *Lactobacillus plantarum* (Nissen et al. 2005; Ladero et al. 2007). Both homo- and heterofermentative lactic acid bacteria produce mannitol. Generally homofermentative lactic acid bacteria produce little amount of mannitol with the help of mannitol 1-phosphate dehydrogenase enzyme. Such as, in the presence of large amounts of glucose or sucrose, *Streptococcus mutans*, *Lactobacillus leichmanii* (Chalfan et al. 1975), *Lactobacillus plantarum* and *Lactococcus lactis* (Neves et al. 2000) produce mannitol. Hetero fer-

mentive lactic acid bacteria produce substantial amount of mannitol with the help of mannitol dehydrogenase enzyme. In the presence of fructose or sucrose, *Leuconostoc (pseudo)mesenteroides* produces high levels of mannitol (Grobben et al. 2001). Two other heterofermentative lactic acid bacteria, *Lactobacillus sp.* and *Leuconostoc sp.* also produced mannitol from fructose and sucrose (Saha and Nakamura 2003). The amount of mannitol produced under optimal culture conditions by *Lactobacillus* and *Leuconostoc* strains were 73 and 26 g/l from 100 g/l fructose with yields of 86% and 65%, respectively (Yun and Kim 1998).

33.9 Future Perspectives

Genome sequencing and functional genomic studies of a variety of LAB are rapidly providing insight into their diversity and evolution and revealing the molecular basis for important traits like flavour formation, sugar metabolism, stress response adaptation and interaction. LAB have been investigated using biotechnological techniques, including genetically modified organisms (GMOs) that contribute to the reliability of food fermentation and better process control. New properties can be introduced that enhance quality, taste, structure and wholesomeness of food and consequently bring benefit to the consumer. Also the use of LAB as probiotics in the treatment of many forms of diarrheal disease appears especially promising. The identification of glucan production gene is important for future challenges for the construction of strains of LAB that produce Glucan with novel properties that could be applied as food additives. The yield of glucans produced by the LAB depends on the composition of the medium, the LAB strain and growth conditions like temperature, pH, oxygen tension and incubation period. The need of the day is to improve the productivity of glucans from LAB, reduce the cost of production for commercial viability and to produce custom made glucans with the desired functionality. The modulation of biochemical properties of glucans require a thorough understanding of its biosynthetic pathway and the relation between the structure of glucans and the functional effect provided by them after incorporation into the food matrix. Several investigators are working on β glucans that can promote antitumor and antimicrobial activity by activating macrophages, dendritic cells and other leukocytes.

33.10 Conclusions

Several metabolites product produced by LAB including organic acids, fatty acids, hydrogen peroxide, carbon dioxide and bioactive peptides have antimicrobial effects. As biopreservatives are more preferred than chemical preservatives, there is an increased interest in prevention of food from spoilage through these metabolites produced by LAB because of their safe association with human fermented foods. The exopolysaccharides that are produced by LAB contribute to the specific rheology and texture of fermented milk products and also have applications in nondairy

foods. When added to food products, polysaccharides function as thickeners, stabilizers, emulsifiers, gelling agents and water binding agents. Several oligosaccharides produced by LAB can act as prebiotic, increase the production of short chain fatty acid. Probiotic LAB strains might play a considerable role during *in vivo* interactions occurring in the human gastrointestinal tract, for instance towards *H. pylori*, *E. coli* and *Salmoella*. Bacteriocin-producing starter or co-cultures have been successfully produced on pilot-scale experiments in making cheese, fermented sausage, sourdough, etc., yielding food quality with food safety advantages.

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