Lactic Acid Bacteria

F Mozzi, Centro de Referencia para Lactobacilos (CERELA)-CONICET, San Miguel de Tucumán, Argentina

Introduction

Taxonomy and Metabolism of Lactic Acid Bacteria

Lactic acid bacteria (LAB) are a heterogeneous group of phylogenetically closely related microorganisms that produce lactic acid as the major or sole product of carbohydrate fermentation. LAB are Gram positive, nonsporulating, catalase-negative, acid tolerant, nonrespiring but aerotolerant, usually nonmotile cocci or rods with low Guanidine + Citosine (G + C) content. Except for a few species belonging to the *Streptococcus*, *Enterococcus*, *Lactococcus*, and *Carnobacterium* genera, LAB are nonpathogenic with a generally recognized as safe (GRAS) or food-grade status.

LAB have complex nutritional requirements for amino acids, peptides, nucleotide bases, vitamins, minerals, fatty acids, and carbohydrates, components that are usually present in the natural niches they inhabit. These microorganisms are found in a plethora of environments such as plant materials and dairy-, meat-, and cereal-based (fermented) foods, the animal gastrointestinal tract and vagina, and in soil and water. Their ability to inhabit different niches is trait of their metabolic diversity and adaptability.

The monograph on LAB written by Orla-Jensen in 1919 greatly affected the systematics of these microorganisms; LAB were classified based on their morphology, mode of glucose fermentation, ability to grow at different temperatures, and use of sugars as carbon substrates. Other features, such as configuration of lactic acid produced and tolerance to salt, acid, and alkali, were also considered. According to the current taxonomic classification LAB belong to the phylum Firmicutes, class Bacilli, order *Lactobacillales*. The families include Aerococcaceae, Carnobacteriaceae, Enterococcaceae, Lactobacillaceae, Leuconostocaceae, and Streptococcaceae. Four main genera of LAB, namely *Lactobacillus*, *Leuconostoc*, *Pediococcus*, and *Streptococcus*, were originally described; however, recent taxonomic revisions have proposed the following new genera: *Aerococcus*, *Alloiococcus*, *Carnobacterium*, *Dolosigranulum*, *Enterococcus*, *Globicatella*, *Lactococcus*, *Oenococcus*, *Tetragenococcus*, *Vagococcus*, and *Weisella*. Of all the genera mentioned, the lactobacilli and carnobacteria are rods; the remaining genera are cocci, except for *Weisella* species, which may be either rods or cocci. Researchers classified some of the newly described genera using fatty acid composition and motility features.

With the availability of improved molecular methodologies such as 16S rRNA gene sequencing and studies on the microbial diversity of different environments, many species have been discovered and identified. Thus, the heterogeneous LAB group consists of about 450 species distributed as follows: *Aerococcus*, 7; *Alloiococcus*, 1; *Carnobacterium*, 12; *Dolosigranulum*, 1; *Enterococcus*, 49; *Globicatella*, 2; *Lactobacillus*, 189; *Lactococcus*, 7; *Leuconostoc*, 23; *Pediococcus*, 15; *Oenococcus*, 2; *Streptococcus*, 101; *Tetragenococcus*, 5; *Vagococcus*, 8; and *Weisella*, 18.

LAB are defined as chemotrophic microbes as they generate energy (ATP) by substrate-level phosphorylation. Although these bacteria ferment sugars when they grow under anaerobic conditions, they can also grow in the presence of oxygen. Despite their well-established fermentative metabolism, several LAB species can switch from fermentation to aerobic respiration metabolism when heme or heme and menaquinone groups are provided. Electron transport chains and evidence of cytochromes in heme-grown cultures were reported first in lactococci, leuconostocs, and some enterococci. Interestingly, it has been observed that, in *Lactococcus lactis*, respiration has a positive effect as bacterial biomass increases together with oxygen resistance and survival.

Sugar metabolism

Sugars are the primary carbon and energy sources for LAB grown on substrates used for fermented foods and feed production, as well as in laboratory media. According to the way LAB ferment carbohydrates, they are classified as homofermentative or heterofermentative. Homofermentative LAB (*Lactococcus*, *Streptococcus*, *Pediococcus*, *Enterococcus*, and some *Lactobacillus* species) ferment sugars by the Embden–Meyerhoff–Parnas pathway to pyruvate, which is converted into lactic acid by lactate dehydrogenase (LDH). Two types of lactate isomers, L and D, can be produced by stereospecific nicotinamide adenine dinucleotide (NAD)-dependent enzymes, L-LDH and D-LDH. Under certain growing conditions such as carbon limitation, the homolactic metabolism can be shifted to a mixed-acid metabolism where formate, acetate, ethanol, and/or CO_2 besides lactate, are produced (Figure 1).

Heterofermentative LAB such as *Leuconostoc*, *Oenococcus*, and certain *Lactobacillus* species ferment sugars generally by the phosphoketolase pathway (PKP, also known as the pentose phosphoketolase shunt or the 6-phosphogluconate pathway). Fermentation of pentoses such as xylose and ribose leads to the formation of pyruvate and acetyl-P and their subsequent conversion to lactate and acetate, respectively. Hexoses (i.e., glucose, fructose, and mannose) can be converted to lactate, CO_2, and ethanol. CO_2 is a product of 6-P-gluconate degradation, which occurs during conversion of hexoses to pentoses. The specific enzyme of the heterofermentative pathway is D-xylulose-5P phosphoketolase, which catalyzes the conversion of xylulose-5P into glyceraldehyde-3-phosphate (GAP) and acetyl-P.

The fermentation type (homolactic or heterolactic) is an important taxonomic criterion. The genera *Leuconostoc*, *Oenococcus*, and *Weisella* as well as certain *Lactobacillus* species such as *Lactobacillus buchneri*, *Lactobacillus brevis*, *Lactobacillus fermentum*, and *Lactobacillus reuteri* are obligate heterofermentative. Other lactobacilli, including *Lactobacillus acidophilus*, *Lactobacillus delbrueckii*, *Lactobacillus helveticus*, and *Lactobacillus salivarius*, are obligate homofermentative and not able to metabolize pentoses. Finally, *Lactobacillus casei*, *Lactobacillus curvatus*, *Lactobacillus plantarum*, *Lactobacillus sakei*, and most LAB can homofermentatively ferment hexoses and pentoses and are known as *facultatively heterofermentative*.

 http://dx.doi.org/10.1016/B978-0-12-384947-2.00414-1

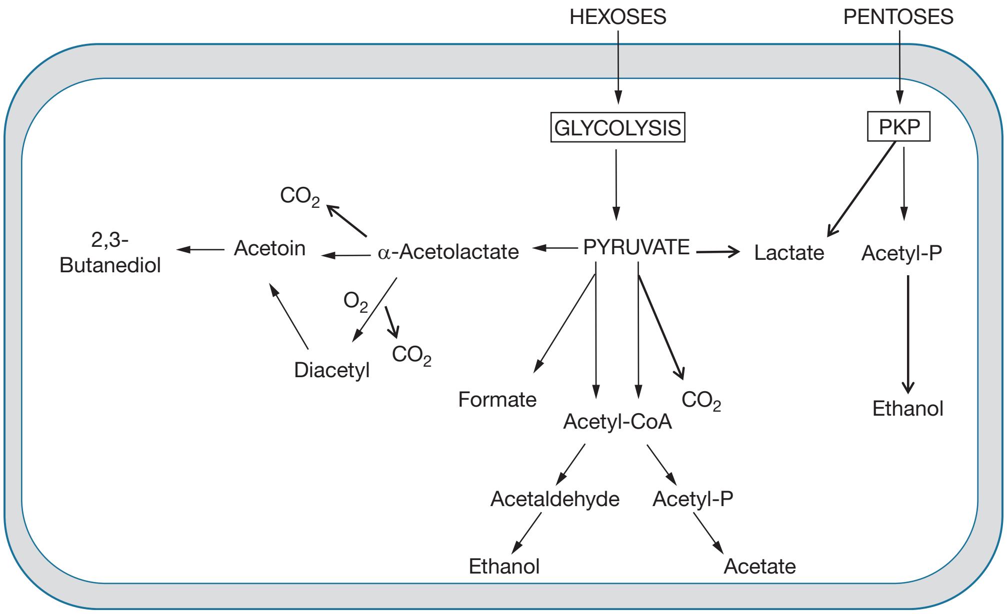

Figure 1 Sugar metabolism in LAB.

Pyruvate has a key role in the fermentation pathways; it usually acts as an electron acceptor to form lactic acid and to help maintain the intracellular redox balance. Nevertheless, and depending on the LAB species, pyruvate can be metabolized by different enzymes leading to the synthesis of different metabolites such as diacetyl/acetoin, formate, and acetyl-coA, which can subsequently yield ethanol or ATP and acetate. Also, oxygen and fructose may be used as alternative electron acceptors rather than pyruvate.

In addition to being capable of fermenting several monosaccharides, LAB can also use disaccharides as energy substrates. In this regard, lactose metabolism has been extensively studied, especially in those species commonly used in dairy applications. In this case, lactose can enter the cell through a lactose-specific permease or a lactose-specific phosphoenolpyruvate–phosphotransferase (PEP–PTS) system or both. In the first system, lactose is cleaved into glucose and galactose by a beta-galactosidase enzyme, which in turn can be fermented through the main fermentation pathways. If lactose enters the cells by a PEP–PTS system, lactose-P is split into glucose and galactose-6-P by the enzyme phospho-beta-galactosidase. On the other hand, the disaccharides maltose (present in barley) and sucrose (present in vegetables and fruits) can also be used by certain LAB members.

Nitrogen metabolism

Many LAB have a very limited capacity to synthesize amino acids from inorganic nitrogen sources so they depend on the availability of amino acids present in the environment or growth medium. Dairy LAB highly depend on their proteolytic system, which in turn can degrade external proteins and transport the resulting peptides and amino acids into the cell. The proteolytic system of LAB has been intensively studied, especially in model organisms such as dairy lactococci but also in many lactobacilli such as *Lactobacillus helveticus* and *Lactobacillus delbrueckii* subsp. *lactis*.

Degradation of casein, the major milk protein, is dependent on a cell wall-associated proteinase; this protease hydrolyzes casein into oligopeptides of varied sizes that in turn enter the cell by different transport systems. Inside, peptides are further degraded into amino acids for microbial metabolism and growth.

Applications of LAB

Microbial fermentation increases the shelf-life of perishable raw materials and imparts a characteristic flavor to the fermented products. In addition, fermentation by beneficial microorganisms often introduces or increases health benefits.

Since ancient times, LAB have been empirically used in the preservation and production of fermented foods of plant or animal origin. Since the 1930s and 1940s, LAB have been used as lactic starter cultures in the fermented food industry. Later, because of increased knowledge in the immunology field, these microorganisms started to be used as probiotics, while much more recently efforts have been made to use LAB as microbial cell factories for the production of interesting metabolites. These applications are discussed later in the section 'LAB as Microbial Cell Factories'.

LAB as Starter Cultures in the Food Industry

LAB, the so-called powerhouse or workhorse microbes of the fermented food industry, are intensively employed in food and feed fermentation as well as in food biotechnology.

The ability of LAB to produce lactic acid from various sugars plays an important role in food fermentation. As lactic acid derives from pyruvate (a glycolysis end product), a fast and desirable lactic acid production rate requires a high glycolytic flux. In addition to lactic acid, other alternative end products such as ethanol, acetic acid, and formic acid are formed by many species. LAB have the ability to ferment a variety of substrates from dairy, meat, vegetables, and cereals, transforming them into tasty products with unique textures and improved (longer) shelf lives. Depending on the raw material, specific, niche-adapted LAB genera and species are employed to carry out fermentation to produce a variety of fermented foods and beverages such as yogurts, fermented milks, buttermilk, kefir, cheeses, fermented sausages, fermented fish, sourdough, pickles, olives, sauerkraut, and other fermented vegetables and fruits. Examples of such fermented foods and beverages and the LAB involved are shown in Table 1.

Despite the desirable role of LAB in food fermentation, some psychrotrophic species (i.e., *Leuconostoc mesenteroides*, *Leuconostoc gasicomitatum*, *Lactobacillus curvatus*, and *Lactobacillus sakei*) have been reported to cause detrimental effects in packaged (air, vacuum, and modified-atmosphere) foods. Souring, strange flavors, gas formation, discoloration, slime production, and decreased pH are some defects caused by the presence of this type of bacteria in ready-to-eat vegetable and fruit salads, fresh raw meat, cooked meat products, and composite foods.

Lactic starter cultures are defined as actively growing cultures of LAB that are used to start and control the fermentation process; in addition, these microorganisms contribute to the sensory characteristics of the fermented products and to their safety. Even today, the most relevant industrial application of LAB is their use as starter cultures in food fermentation. Because the starter industry relies on the use of selected strains of certain species with known metabolic properties, the use of starter cultures has undoubtedly improved the commercial and hygienic quality of the fermented final products and the standardization of the entire process. In contrast, the limited number of available strains with high technological performance and the risk of bacteriophage attack results in the search for new strains to diversify products. In this respect, artisanal or homemade fermentations represent a rich source of indigenous LAB for commercial use. Currently, defined, multi-, or mono-strain cultures prepared as frozen or freeze-dried cell concentrates are commercially available.

During the past few decades because of the huge worldwide industrial market for lactic fermentation (estimated as more

Table 1 Examples of fermented foods and beverages using LAB

Fermented foods and beverages	*LAB species*
Dairy products	
Yogurt	*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*
Fermented milks	*Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, *Lactobacillus johnsonii*, *Lactobacillus reuteri*
Kefir	*Lactobacillus kefir*, *Lactobacillus kefiranofaciens*, *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Lactobacillus kefiri*
Buttermilk	*Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *lactis* var. *diacetylactis*
Cheeses	*Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris*, *Leuconostoc mesenteroides* subsp. *cremoris*, *Lactobacillus delbrueckii* subsp. *lactis*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus helveticus*, *Streptococcus thermophilus*
Meat products	
Fermented sausages	*Lactobacillus sakei*, *Lactobacillus curvatus*, *Pediococcus acidilactici*
Fish products	
Fermented fish	*Lactobacillus alimentarius*, *Carnobacterium piscicola*
Vegetable products	
Olives	*Pediococcus acidilactici*, *Pediococcus parvulus*, *Leuconostoc mesenteroides*, *Leuconostoc pseudomesenteroides*, *Lactobacillus plantarum*, *Lactobacillus pentosus*
Sauerkraut	*Pediococcus pentosus*, *Leuconostoc mesenteroides*, *Lactobacillus plantarum*
Leeks	*Leuconostoc mesenteroides*, *Lactobacillus plantarum*, *Lactobacillus sakei*
Eggplant, mustard, beets, peppers, tomatoes, carrots, capers, cabbage	*Lactobacillus fermentum*, *Lactobacillus pentosus*, *Lactobacillus plantarum*, *Pediococcus pentosaceus*, *Lactobacillus brevis*, *Lactobacillus paracasei*, *Lactobacillus pantheris*, *Pediococcus acidilactici*, *Lactobacillus curvatus*, *Weisella consufa*, *Weisella soli*, *Enterococcus faecium*
Soybeans	*Leuconostocs*, *Lactococcus lactis*
Fermented fruits	
Pineapples	*Lactobacillus plantarum*, *Lactobacillus rossiae*
Kiwis	*Lactobacillus plantarum*
Papayas	*Lactobacillus plantarum*, *Lactobacillus pentosus*, *Weisella confusa*, *Weisella cibaria*
Cherries	*Lactobacillus plantarum*, *Leuconostoc mesenteroides* subsp. *mesenteroides*, *Pediococcus pentosaceus*
Alcoholic beverages	
Wine	*Oenococcus oeni*
Rice wine	*Lactobacillus sakei*, *Pediococcus acidilactici*
Cereal-based products	
Sourdough	*Lactobacillus sanfranciscensis*, *Lactobacillus farciminis*, *Lactobacillus fermentum*, *Lactobacillus brevis*, *Lactobacillus plantarum*, *Lactobacillus amylovorus*, *Lactobacillus reuteri*, *Lactobacillus pontis*, *Lactobacillus panis*, *Lactobacillus alimentarius*, *Pediococcus pentosaceus*, *Weisella cibaria*
Fermented maize products	*Lactobacillus fermentum*, *Lactobacillus plantarum*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus helveticus*, *Lactococcus lactis* subsp. *cremoris*

than 100 billion euros in 2011), especially in Western countries, many efforts have been made to improve the performance of LAB as starter cultures or to better understand their physiology for the production of industrially interesting metabolites. As a result, innovation is leading to the commercialization of more stress-tolerant, phage-resistant strains as well as starter cultures that lead to improved organoleptic properties. In this context, some metabolic traits of lactic starter cultures of technological interest, such as production of aroma compounds, bacteriocins, exopolysaccharides (EPS), low-calorie sugars, and so on, have been the targets of research and will be discussed later in this article.

LAB metabolism results in both decreased carbohydrate content in the raw material they ferment and decreased pH caused by lactic acid production. As mentioned earlier, this rapid acidification process is one of the most desirable effects of LAB growth, enabling the inhibition of detrimental microorganisms including several common human pathogens, and thus prolonging the shelf lives of fermented products. While lactic acid imparts a distinctive, desirable, and fresh acid taste to fermented products, the fermentation process must be controlled to avoid excessive acid concentration, which may be rejected by consumers and may mask more delicate flavors such as diacetyl. In cheese making, lactic acid production is also responsible for milk coagulation and texture properties. The pH decrease indirectly affects flavor by controlling the proteolytic activity of both coagulant and natural milk proteinases and by influencing the biochemical reactions involved in the formation of other aroma compounds. In this respect, LAB may enhance the organoleptic properties of the fermented product by imparting a characteristic flavor, which may derive from the fermentation of lactose or other sugars, citrate, the degradation of milk proteins and fat, and the metabolism of amino acids and free fatty acids. The flavor of the final product depends on several factors such as composition of the starter culture and raw material, type of fermentation, and ripening and storage conditions.

Besides the contribution of LAB to the flavor of fermented foods, some strains may contribute to the texture and nutritional quality of the final product. In addition, some LAB secrete natural food preservatives because of the production of antifungal and antibacterial compounds such as additional carboxylic acids (i.e., phenyl-lactic acid and acetic acid), fatty acids, ethanol, carbon dioxide, hydrogen peroxide, and bacteriocins.

Bacteriocin production is a desirable feature because they help control microbial populations in fermented foods and, thus, extend product shelf-life and safety. Bacteriocins produced by LAB are a diverse group of ribosomally synthesized antimicrobial peptides, which may be classified into different groups depending on the presence of posttranslational modifications and if they are active as single- or two-peptide bacteriocins. Nisin is a broad spectrum bacteriocin, which inhibits Gram-positive foodborne pathogens and spoilage microbes as well as Gram-negative bacteria if combined with additional preventatives. Nisin has been the paradigm, the most studied and commercially applied bacteriocin to date. Narrow spectrum bacteriocins, such as lactococcin A, can also be of value as these bacteriocins have lytic effect on sensitive lactococci and may release key enzymes that can accelerate cheese ripening and enhance the development of important organoleptic properties. In food, bacteriocins may be present via in situ production by bacterial fermentation, by the addition of purified or semipurified preparations, or as an ingredient based on a fermentate of a bacteriocin-producing strain.

Certain LAB strains are able to produce EPS, which are polymers of carbohydrates. Depending on their chemical compositions, EPS can be classified as homopolysaccharides, which contain a single type of monosaccharide, and heteropolysaccharides, which are formed from several repeating units of different monosaccharides. The ability to synthesize EPS is widespread among LAB species; however, a wide diversity in respect to EPS structures and to the amount of polysaccharides produced exists among the LAB producer strains. The inclusion of EPS-producing strains in starter cultures is desirable as these biopolymers naturally confer suitable technological characteristics to dairy products, such as improvement of rheological properties and reduction of syneresis of fermented milks as well as better consistency of curd in low-fat ripened cheeses. In addition, health-promoting effects such as a possible role as prebiotics, serum cholesterol-lowering ability, and immunomodulatory and anticarcinogenic activities have been ascribed to EPS produced by LAB. Conversely, the presence of EPS in wine and beer is detrimental.

LAB as Microbial Cell Factories

The central role of glycolysis in LAB has been at the basis of numerous studies to identify potential bottlenecks of the flux control needed not only for optimizing food fermentation processes but also for novel applications of LAB, such as their use as cell factories for the production of food ingredients, green fuels, and chemicals. In this respect, LAB are being used as 'cell factories' for the production of certain metabolites either to be used as purified compounds or to be produced in situ in fermented foods; examples include the production of bacteriocins, vitamins, amino acids, and low-calorie sugars. The use of LAB as cell factories is mainly because of their food-grade status and beneficial effects in the gastrointestinal tract, the availability of genetic tools for strain improvement, the possibility of using cheap substrates for growth, and direct application of their metabolites in food products. It can be affirmed that LAB applications have currently moved beyond the traditional food fermentation processes to the use of LAB as delivery vehicles for drugs, as vaccines, and as microbial cell factories for industrially relevant metabolites.

Metabolic engineering approaches in LAB were first reported in 1995 in an attempt to deviate pyruvate formation in the production of the buttery aroma compound diacetyl by *Lactococcus lactis*. Gene manipulation techniques together with some features of LAB such as small genomes (1.8–3.4 Mbp), fast growth, high sugar update rates, and a relatively simple metabolism, allowed the generation of efficient microbial cell factories. In the past few years, *Lactococcus lactis* and certain *Lactobacillus* species have been manipulated to synthesize metabolites such as lactate, 1,3-propanediol (1,3-PDO), biofuels, food flavors, sweeteners, and EPS. More recently, efforts are being directed to the production of commodity compounds and chemicals with applications in medicine (Table 2).

Table 2 Use of LAB as microbial cell factories for the synthesis of industrially interesting metabolites

Type of metabolite	*Compounds*	*Applications*
High added-value compounds	Lactic acid	Food additive and food preservative; used in food and plastic industries
	2,3-Butanediol (2, 3-BDO)	Antifreeze in plastic and other industrial processes
	1,3-Propanediol (1, 3-PDO)	Polymer, solvent, and cosmetic industries
	Ethanol	Biofuel alternative to gasoline
	Butanol	Solvent; alternative biofuel
Food ingredients	Alanine	Low-calorie sweetener in food
	Diacetyl	Aroma compound in food
	Acetaldehyde	Aroma compound in food
	Vanillin	Flavoring compound in food
Nutrients	Polyols (mannitol, sorbitol, erythritol, etc.)	Low-calorie sugars and sugar replacers in food industry; in medicine, used as a potent osmotic diuretic
	Vitamins (B_{12}, folate, riboflavin)	Essential nutrients in food
	Short-chain fatty acids (SCFAs)	Beneficial health effects
	Conjugated linoleic acids (CLAs)	Beneficial health effects
	Bioactive peptides	Antihypertensive, antioxidant, antimutagenic, etc.
	Gamma-aminobutyric acid (GABA)	Relaxing effect on gut muscles

High added-value compounds

Recently, the production of high added-value chemicals by LAB has been reported, including lactic acid, 2,3-butanediol (2,3-BDO), 1,3-PDO, bioethanol, and butanol.

Lactic acid has been used in the food industry as a flavor additive and food preservative; however, its main use in the market is in the creation of biodegradable plastics (as a precursor of poly-lactic acid) and pharmaceuticals. Traditionally *Lactobacillus casei* has been used to produce lactic acid because of its ability to convert more than 95% of the available sugar into this organic acid. However, by means of metabolic engineering processes and the use of renewable resources such as agro-industrial wastes as substrate, other species such as *Lactobacillus helveticus* and *Lactobacillus plantarum* have been able to efficiently form lactic acid either by achieving the deletion of the *L-ldh* (lactate dehydrogenase) gene or by introducing the *xyl*AB operon of *Lactobacillus pentosus* in the *Lactobacillus plantarum* genome. By these means, production of pure D-lactic acid with a 0.8 $g\,g^{-1}$ yield was achieved.

Another important chemical compound is 2,3-BDO which is used as an antifreeze in the plastic, chemical, pharmaceutical, and cosmetic industries. Moreover, acetoin and diacetyl, important buttery flavors in some food products, can be formed by dehydrogenation of 2,3-BDO. Biotechnological processes have produced 2,3-BDO using *Klebsiella* strains; however, the pathogenic status of this bacterium has encouraged its synthesis by LAB members because many species possess the required biosynthetic pathway. In this respect engineered strains of *Lactobacillus plantarum* and *Lactococcus lactis* have been shown to efficiently produce 2,3-BDO from inexpensive substrates such as whey permeate.

1,3-PDO is another compound with multiple applications in the production of polymers, solvents, resins, detergents, and cosmetics. To date, this diol is industrially produced by chemical synthesis; however, its microbial production from glycerol, a byproduct of the biodiesel industry, has been intensively studied. Again, the food-grade status of LAB makes it the preferred option for synthesis of 1,3-PDO, rather than *Klebsiella* and *Clostridium* strains, which are considered opportunistic pathogens and thus are not applicable for food and biomedical products. Among the naturally lactobacilli producers of 1,3-PDO, *Lactobacillus reuteri* has been reported to be the best one so far.

Biotechnological production of ethanol may be one of the most important sources of renewable energy because this compound is used as an alternative biofuel to gasoline. In the past few years, to overcome the natural restrictions of crude oil use, researchers have become interested in using LAB to produce ethanol from renewable biomass such as lignocellulose, given that these microorganisms can metabolize different sugars. So far, the most promising results have been reported for an LDH-negative strain of *Lactobacillus plantarum*, that expresses pyruvate decarboxylase from a strain of the Gram-positive *Sarcina ventriculi*.

Also, butanol is a desirable solvent that may be an attractive fuel alternative. While butanol-producing strains cannot tolerate amounts higher than 2%, researchers may apply genetic engineering strategies to certain LAB members to produce butanol in the presence of 3% butanol.

Food ingredients/additives

Several compounds formed by LAB, such as alanine, diacetyl, and acetaldehyde, are currently employed as food ingredients.

Alanine is an amino acid with a natural sweet taste that is used as a food and pharmaceutical additive. It is produced through pyruvate reduction by alanine dehydrogenase in the presence of ammonium. Homoalanine fermentation is achieved by a metabolic engineering strategy through the heterologous overexpression of alanine dehydrogenase from *Bacillus sphaericus* in an LDH-deficient *Lactococcus lactis* strain.

Diacetyl is an important flavor compound that has a buttery aroma and is typically used for dairy products; diacetyl is naturally produced by LAB from citrate, which limits its production because citrate availability in raw materials is usually low. To surpass this limitation, researchers have created some metabolic engineering strategies to produce diacetyl from lactose or glucose.

Acetaldehyde is an important aroma compound, especially in dairy products; for instance, it is the main aroma compound in yogurt. Because acetaldehyde is an intermediate metabolite from ethanol metabolism, efforts to increase diacetyl production are usually connected with the ethanol pathway. So far, the best engineered strategy for this purpose consists of the heterologous overexpression of pyruvate decarboxylase from *Zymomonas mobilis* with native NADH oxidase in *Lactococcus lactis*.

Vanillin is one of the most used flavoring compounds worldwide; as the annual world market demand of vanillin cannot be met by natural extraction, chemical synthesis or tissue culture biotechnological approaches have been considered. Biovanillin synthesis using GRAS LAB and metabolically engineered microorganisms is currently being studied.

Low-calorie sugars

Polyols, or low-calorie sugars, are sugar alcohols that naturally occur in nature; among them, mannitol and erythritol are the only ones naturally produced by LAB. These two sugar alcohols, together with sorbitol and xylitol, are widely used in the food and pharmaceutical industries and in medicine because of their interesting physicochemical properties. Moreover, as these low-energy, nonmetabolizable sweeteners do not affect insulin levels, they are employed in dietetic and diabetic food products.

In the past decade, biotechnological production of polyols by LAB has been sought as an alternative to their current chemical production. While heterofermentative LAB may naturally produce mannitol and erythritol under certain culture conditions, sorbitol and xylitol have been synthesized only through metabolic engineering processes. Although strains belonging to the *Oenococcus*, *Leuconostoc*, and *Weisella* genera have been reported to form mannitol, the best mannitol-producing microbes reported so far are heterofermentative lactobacilli such as *Lactobacillus fermentum* and *Lactobacillus reuteri*. Controlled culture conditions using selected LAB strains have yielded amounts of mannitol that are similar to those of current industrial chemical processes.

Sorbitol production by *Lactobacillus casei* and *Lactobacillus plantarum* strains has been achieved by reverting the sorbitol catabolic pathway, taking advantage of the reversibility of the enzyme sorbitol 6-P dehydrogenase, which is responsible for the interconversion of sorbitol 6-P into fructose 6-P.

While metabolic engineering strategies using homologous genes have been employed for sorbitol synthesis by LAB, xylitol production has been achieved only by heterologous expression of D-xylose reductase and xylose transporter genes from the yeast *Pichia stipitis* CBS 5773 and the strain *Lactobacillus brevis* ATCC 8287, respectively.

Nutrients

A wide range of compounds with nutritional relevance such as vitamins, polysaccharides, short-chain fatty acids (SCFAs), conjugated linoleic acids (CLAs), and bioactive peptides are synthesized by LAB.

Vitamins are essential micronutrients for the metabolism of living organisms. Because of insufficient food intake or unbalanced diets, human vitamin deficiencies are still very common. As an attractive alternative to chemical vitamin synthesis, biotechnological production of vitamins using LAB had been under study in the 2000's. Furthermore, fermentation with food-grade LAB offers unique opportunities to improve the nutritional value of food products and the development of novel functional foods with enhanced vitamin content. As a result of secondary metabolism, certain LAB are able to synthesize some group B vitamins such as B_{12} (cobalamin), B_{11} (folate), and B_2 (riboflavin). In this respect, several industrially important LAB such as *Lactococcus lactis* and *Streptococcus thermophilus* are capable of synthesizing folate as has been observed in fermented milks with high vitamin levels. Riboflavin's biosynthesis has been identified in several species, and some applications of riboflavin-producing LAB in dairy and cereal-based products have also been reported. Cobalamin, a complex corrin compound, was found to be produced for the first time by *Lactobacillus reuteri*, a well-recognized probiotic species. In general, vitamin production by LAB varies considerably because it is a species-specific or strain-dependent trait. This feature is generally related to the partial or complete interruption of the genetic information for vitamin biosynthesis. The use of vitamin overproducer LAB strains may be exploited for their in situ production of fermented products.

SCFAs are not produced anabolically in LAB but rather as the final catabolites of the energy metabolism. The most studied SCFA are butyrate, propionate, and acetate. Besides the SCFA acidifying desirable properties, acetate may be metabolized by muscles and other tissues when entering the peripheral circulation while propionate is taken up by the liver. Both acids can lower glycemia, improving insulin sensitivity. Conversely, butyrate has been claimed to have detrimental effects on body weight and obesity because it represents an additional energy source. In contrast, butyrate may control oxidative and metabolic stress at the molecular level by enhancing repair responses and inhibiting the synthesis of pro-inflammatory (IL12, TNF, gamma IF) cytokines.

CLAs are a novel type of biologically beneficial functional lipids. Human metabolism can also be affected by these compounds as some CLA isomers may reduce cancer cell viability and ameliorate insulin resistance while others may lower the HDL/LDL ratio and modify eicosanoid production, with detrimental health effects. So far, the best CLA-producing strains belong to the genera *Lactobacillus* and *Streptococcus*. The most frequently found isomers of linoleic acid have the unsaturation site at positions 9, 11 or 10, or 12, namely, Δtrans 9-, cis11-octa decadienoic acid, and, in lower amounts, Δcis10-, trans12-linoleic acid. CLAs could have health beneficial effects on inflammation, cancer, metabolic disorders, and cardiovascular diseases. However, the beneficial effects of CLAs depend on the isomers and doses.

Bioactive peptides are hidden peptides, which are encrypted in the latent state within the primary sequence of proteins that require enzymatic proteolysis for their release. Bioactive peptides can be produced during gastrointestinal digestion or food processing. LAB that have reduced amino acid biosynthetic abilities harbor a sophisticated proteolytic system, which may contribute to the release of bioactive peptides or their precursors. Thus, proteolytic LAB strains used as starter cultures in the manufacture of different foods (e.g., yogurts, fermented milks, and cheeses) may have a positive effect in this sense. Diverse health benefits have been ascribed to bioactive peptides derived from LAB, including antimicrobial, immunomodulatory, antithrombotic, opioid, and antioxidant effects as well as enhancement of mineral absorption and/or bioavailability and blood pressure-lowering activities. Today some milk-derived peptides are commercially produced and used as dietary supplements in functional foods, personal care products, or drugs.

Among other compounds, some LAB strains biosynthesize gamma-aminobutyric acid (GABA), which has a relaxing effect on gut smooth muscles, and beta-phenylethylamine, which contributes to the control of satiety and mood.

LAB as Probiotics

The gut microbiota is a highly diverse and relative stable ecosystem that is being increasingly recognized for its impact on human health. Deviation from its normal composition is usually associated with localized and systemic diseases. Modulation of the gut microbiota may help to improve health and can be achieved by different nutritional concepts, varying from specific food ingredients to complex diets or by the ingestion of particular live microorganisms such as probiotics.

Probiotics have been defined as "live microorganisms that when administered in appropriate dose, they confer a health benefit to the host" (FAO/WHO 2001). Probiotics have been empirically used for centuries, but only in recent decades has their contribution to the modulation of immunological, respiratory, and gastrointestinal functions started to be fully appreciated and scientifically evaluated. To date, the majority of the available commercial probiotics are LAB, mainly of the *Lactobacillus* genera but also bifidobacteria (a group of bacteria with a high G+C content included in the phylum Actinobacteria that also produce lactic acid but always in combination with acetic acid). A much smaller number of leuconostocs, pediococci, lactococci, enterococci, and streptococci are also used as probiotics.

Probiotic strains are usually isolated from traditional fermented products and the gut, feces, and breast milk of human subjects. The identification of microorganisms is the first step in the selection of potential probiotics. The ability to examine fully sequenced genomes has accelerated the application of genetic approaches to elucidate the functional roles of probiotics.

Fermented foods and beverages possess various nutritional and therapeutic properties; in this sense LAB play a major role in the positive health effects of fermented milks and related products. Such foods, beverages, and powders are highly acceptable to consumers because of their flavor and high nutritional value. Potential health benefits of fermented milks include antimutagenic and antitumor activity (associated with the cell wall of starter bacteria), prevention of gastrointestinal infections, and reduction of serum cholesterol levels. Although probiotics are mainly used for gastrointestinal benefits, from which the best-demonstrated clinical benefits of probiotics are the prevention and treatment of antibiotic-associated diarrhea, their use can also extend to skin, oral, and vaginal health, as well as the treatment of allergies, liver disorders, and metabolic diseases (Table 3). Recently, investigation of the gut-brain connection suggested that the gut microbiota play a key role in mood and anxiety

Table 3 Examples of beneficial health properties of LAB used as probiotics

Beneficial health effect	*Associated LAB species*
Antidiarrheal	*Lactobacillus acidophilus*
Antitumor activity	*Lactobacillus reuteri*
Antimutagenic activity	*Lactobacillus casei*
Anticarcinogenic effects	*Lactobacillus paracasei*
Serum cholesterol reduction	*Lactobacillus rhamnosus*
Prevention of gastrointestinal infections	*Lactobacillus kefir*
Improvement of the gut immune barrier	*Lactobacillus plantarum*
Modulation of the immune response	*Lactobacillus helveticus*
Treatment of allergy disorders	*Lactobacillus delbrueckii* subsp. *lactics*
Regulation of the gut and vagina microbiota	*Lactobacillus delbrueckii* subsp. *bulgaricus*
	Lactobacillus gasseri
	Lactobacillus johnsonni
	Lactococcus lactis
	Lactobacillus salivarius
	Lactobacillus pentosus
	Streptococcus thermophilus
	Enterococcus faecalis
	Enterococcus faecium
	Pediococcus acidilactici
	Pediococcus pentosaceus

disorders, supporting the inclusion of probiotics in animal and human products. It has been suggested that an improvement of the intestinal barrier function through microbiota modification can decrease lipopolysaccharide infiltration, reduce inflammatory parameters in the fat tissue, and, consequently, reduce the chances of developing obesity and insulin resistance. Probiotics may also regulate host intestinal microbiota, improve gut immune barrier function, and achieve a balance between pro-inflammatory and anti-inflammatory cytokines.

Probiotic bacteria typically colonize the intestinal tract first and then reinforce the host defense systems by inducing generalized mucosal immune responses, including modulation of dendritic cell/natural killer interaction, a balanced T-helper cell response, self-limited inflammatory response, and the secretion of polymeric Immunoglobulin A. Many reports have demonstrated that LAB, mainly *Lactobacillus* as well as *Bifidobacterium* and their fermented products, may improve innate and adaptive immunity, prevent gastric mucosal lesions, alleviate allergies, and create a defense against intestinal pathogen infection. Also, probiotic LAB have been included in the production of functional foods with specific properties in order to potentially prevent or treat a range of intestinal maladies including inflammatory bowel disease, constipation, and colon cancer.

It has been shown that fermented animal feed using LAB has been beneficial in pig nutrition and the nutrition of other farm animals as a tool to reduce gut microbial disorders, to increase antibacterial activity, and to enhance immunity. Colonization of the digestive tract in animals begins immediately after birth because adult microbiota dramatically change during the host's life. In farm animals, the role of the gut microbiota, which is similar to that in humans, does not differ much among animal species. To ensure the animals optimal growth, production, and health, the microbiota of the animal gastrointestinal tract can be manipulated by probiotic bacteria. Probiotics have already been given to different farm animals such as cattle, chicks, piglets, lambs, and small ruminants. In this respect, LAB have been proposed as protective bacteria to reduce *Escherichia coli* O157 in cattle and *Salmonella* in poultry, as well as to prevent diarrheal disease in piglets.

Conclusions

Here I have presented information about the versatility of the heterogeneous, broad LAB group, including findings about their metabolism, food and industrial applications, and health benefits. Their traditional and empirical use in food preservation from the time of the earliest written words has evolved beyond imagination along with scientific progress. This long and challenging journey has allowed not only the development of rational and novel applications of LAB but also a deeper understanding of the involved mechanisms to improve human health and well-being.

See also: Fermented Foods: Composition and Health Effects; Fermented Foods: Fermented Meat Products; Fermented Foods: Fermented Milks; Fermented Foods: Fermented Vegetables and Other Products; Fermented Foods: Origins and Applications; Fermented Foods: Use of Starter Cultures; Functional Foods; Probiotics; Yogurt: Yogurt Based Products

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