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Chapter 5

Waste Degradation and Utilization by Lactic Acid Bacteria: Use of Lactic Acid Bacteria in Production of Food Additives, Bioenergy and Biogas

Galina Novik, Olga Meerovskaya and Victoria Savich

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Abstract

Lactic acid bacteria (LAB) are one of the most well-studied bacterial groups known from ancient times. These valuable microorganisms are used in numerous areas, especially food industry and medicine. LAB produce a wide range of compounds for food upgrading. Moreover, LAB can find special applications like generation of bioenergy not affecting the surrounding environment. The article considers physiological and biochemical processes determining valuable characteristics of the bacteria, potential applications of LAB and their products, especially in food industry and bioenergy sector, and discusses LAB potential contribution into solution of waste disposal problem.

Keywords: lactic acid bacteria, metabolites, waste degradation, food additives, bioenergy

1. Introduction

Lactic acid bacteria (LAB) named so for the appropriate ability to ferment carbohydrates into lactic acid are one of the most studied and used groups of microorganisms. These bacteria have been applied in food processing since ancient times. The first pure culture of LAB was obtained in 1873; however, milk souring and lactic acid producing bacteria were considered as the same microorganisms until the beginning of twentieth century [1]. Today LAB represent a vast and diverse microbial group playing an important role in dairy, baking technology, fish and meat processing. Moreover, LAB are components of normal human microflora and can be used as probiotics to provide health benefits. Thus, LAB find wide applications in food...
industry and medicine. In addition, it is possible to use the bacteria and their products in other fields, such as generation of bioenergy, wood protection, agriculture, bioremediation of environment and so on.

Lactic acid is the main product of LAB synthesis primarily consumed by food industry. These microorganisms are also sources of low calorie sugars, ethanol, aroma compounds, bacteriocins, exopolysaccharides (EPS) and several vitamins utilized in various areas [2].

LAB can be found in any environment rich in carbohydrates. Waste substrates containing these substances, especially food residues, provide an excellent opportunity for LAB cultivation and fabrication of derived products, cost reduction and refuse disposal. Some carbohydrate compounds can be extracted from wastes, like chitin.

This article presents data on taxonomy, identification, physiology and metabolism of LAB, applications of the bacteria and their products, especially in food industry and contribution in production of bioenergy and biogas.

2. Lactic acid bacteria (LAB): taxonomy and identification, physiological and metabolic processes

LAB represent a diverse microbial group united by the ability to produce lactic acid from various substrates. The first pure culture of LAB, now known as *Lactococcus lactis*, was isolated in 1873 by Lister [1]. Originally the term “lactic acid bacteria” denoted “milk souring organisms,” but it came out of use after publication of the monograph by Orla-Jensen (1919) formulating the principles of modern LAB classification [3]. Taxonomic affiliation of the bacteria based on cellular morphology, mode of glucose fermentation, growth temperatures and range of sugar utilization distinguished four core genera: *Lactobacillus*, *Leuconostoc*, *Pediococcus* and *Streptococcus*. The above-mentioned characteristics are still very important for current identification of LAB, despite development of molecular methods. Today LAB are referred to phylum *Firmicutes*, order *Lactobacillales*, genera *Aerococcus*, *Alloioococcus*, *Carnobacterium*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Oenococcus*, *Pediococcus*, *Streptococcus*, *Symbiobacterium*, *Tetragenococcus*, *Vagococcus* and *Weissella*. Sometimes species of *Bifidobacterium* genus (phylum *Actinobacteria*) are assigned to LAB [4]. This genus was distinguished as a separate taxon in 1973 [5]. Until that moment, *Bifidobacterium* was incorporated in other genera, including *Lactobacillus*.

Because of LAB beneficial properties, their correct identification is vital for further industrial and medical use. Phenotypic methods are cheaper compared to genotypic methods, but similar phenotypes displayed by strains do not always correspond to similar or even closely related genotypes. Phenotypic methods also differ by poor reproducibility and ambiguity of some techniques often caused by complex growth conditions, weak discriminatory power and massive arrangements for large-scale studies. Among these methods, protein profiling seems quite reliable for LAB identification. However, even this procedure demands high workload and lacks discriminatory power on the subspecies level, for example, in the *Lactobacillus acidophilus* group [6].
In turn, genotypic methods are not dependent on growth conditions of microorganisms and exhibit various levels of differentiation, from species to individual strains (typing), but they are labor-consuming. Sequencing of the 16S rRNA gene is the most popular molecular tool of identification. Some features make this gene an attractive research object: it is present in all bacteria; 16S rRNA function has remained stable over a long period, so random sequence changes reflect measure of time; the gene is large enough (approximately 1500 bp) to contain statistically significant sequence information [7]. Besides sequencing of 16S rRNA gene, it is possible to carry out hybridization of oligonucleotide probes to reveal taxonomic groups with different specificity from domain to species level. In case of intraspecific identification, other methods are practiced. They include DNA fingerprinting techniques: restriction fragment length polymorphism analysis involving the digestion of genomic DNA with restriction enzymes to large fragments fractionated using pulsed-field gel electrophoresis; randomly amplified polymorphic DNA analysis applying arbitrary primers for amplification of corresponding DNA fragments; amplified restriction length polymorphism method combining two previous techniques and so on. All these methods are successfully used in identification and differentiation of LAB [8].

LAB are Gram-positive rods and cocci characterized by the absence of catalase (although some strains can produce pseudocatalase), tolerance to low pH values and lack of spore formation. These bacteria do not synthesize components of respiratory chains such as cytochromes and porphyrins and cannot generate ATP via proton-gradient mechanism. Therefore, LAB produce ATP predominantly by fermentation of sugars. Because of lack of cytochromes and porphyrins, LAB do not use oxygen, but they can grow in its presence. Protection from oxygen by-products (e.g. H₂O₂) is provided by peroxidases [9].

The distinctive feature of LAB is production of lactic acid. They are chemotrophic microorganisms deriving necessary energy from oxidation of chemical compounds, especially sugars. There are two fermentation pathways: homofermentative and heterofermentative. Homofermentative bacteria produce lactic acid as the major metabolite through glycolysis or Embden-Meyerhof-Parnas pathway generating two moles of lactate per mole of glucose. Pentoses and gluconate are not fermented by microorganisms via obligate homofermentative pathway due to lack of enzyme phosphoketolase. This type of fermentation is inherent, for example, to some species of the genus Lactobacillus (L. acidophilus, L. delbrueckii, L. helveticus, L. salivarius).

In turn, heterofermentative microorganisms using pentose phosphoketolase pathway (hexose monophosphate shunt/6-phosphogluconate pathway) produce equimolar amounts of lactate, CO₂ and ethanol (Figure 1). Genera Leuconostoc, Oenococcus, Weissella and some lactobacilli (L. brevis, L. buchneri, L. fermentum, L. reuteri) are characterized by this type of fermentation. Hexoses other than glucose enter the major pathways after different isomerization and phosphorylation steps [10].

Genus Bifidobacterium differs from LAB by alternative way of sugar conversion known as bifid shunt. Hexoses are degraded through several stages to acetyl-phosphate 2-glyceraldehyde-3-phosphate. The latter is metabolized by Embden-Meyerhof-Parnas pathway to lactic and
acetic acid in the ratio 2:3. This pathway yields 2.5 moles ATP per mole of glucose, whereas homofermentative lactic acid fermentation generates 2 moles of ATP per mole of glucose [11].

Many LAB are able to ferment pentoses. They can digest them heterofermentatively by entering the phosphogluconate pathway as either ribulose-5-phosphate or xylulose-5-phosphate. Pentoses
are converted into lactate and acetate, with no CO₂ evolved [12]. Disaccharides are previously split enzymatically into monosaccharides that enter the appropriate pathways [11].

The proteolytic system of LAB converts proteins to peptides and then to amino acids essential for bacterial growth. The branched-chain amino acids (valine, leucine and isoleucine), the aromatic amino acids (tyrosine, tryptophan and phenylalanine) and the sulfur-containing amino acids (methionine and cysteine) are the main amino acid sources for flavor compounds, such as aldehydes, alcohols and esters, generated using two distinct routes: transamination and elimination [13]. The proteolytic system of LAB includes three major components: cell-wall bound proteinase initiating degradation of extracellular milk protein casein into oligopeptides; transporters taking up the peptides into the cell and various intracellular peptidases degrading the peptides into shorter fragments and amino acids. Components of the proteolytic system are diverse in various groups of LAB as well as in distinct strains. Some enzymes are only found in a few LAB strains, such as cell-wall bound proteinase PrtP. Other ferments, like aminopeptidases PepC, PepN and PepM, and proline peptidases, PepX and PepQ, are represented in all genomes, usually with one gene per genome. It appears logical that bacteria with extensive set of proteolytic enzymes show certain advantages when applied in manufacturing of various compounds [14].

Lipid metabolism proceeds as the breakdown of lipids by lipases into fatty acids and glycerol. LAB are able to produce lipases, but they are less efficient if compared with other microorganisms, such as *Pseudomonas*, *Aeromonas*, *Acinetobacter* or *Candida*, and mostly intracellular. Besides, not all LAB synthesize these enzymes. Only one quarter of lipase-producing strains were detected among 103 tested LAB from the genera *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus* and *Streptococcus*. The majority belonged to *Lactococcus* species [15]. LAB can perform unique fatty acid transformation reactions: isomerization, hydration, dehydration and saturation. Some products of lipid metabolism, e.g. conjugated linoleic acid, can be used for medicinal and nutraceutical purposes [16]. Esterases of LAB are able to catalyze both hydrolysis of fat glycerides with release of free fatty acids and ester synthesis from glycerides and alcohols via transferase reaction. Esterases display the highest activity on monoglycerides, with inferior activity on diglycerides. However, their activities on the specific glycerides and ρ-nitrophenyl or β-naphthyl esters of fatty acids decrease as the carbon-chain length of the esterified fatty acid increases [17].

3. Applications of LAB

LAB are applied in food production and preservation from the ancient times. Nowadays, LAB find wide use in various areas such as synthesis of chemicals and pharmaceuticals or manufacturing of probiotics for agriculture and medicine. Nevertheless, food industry remains to be the domain of broad LAB application. LAB strains were granted “Qualified Presumption of Safety” and “Generally Regarded as Safe” status by the European Food Safety Authority (EFSA) and Food and Agriculture Organization of the United Nations (FAO), respectively. They are used in manufacturing of dairy, meat, baking and vegetable products all over the world [18–21]. These bacteria also allay product allergenicity and ensure longer preservation of fermented foods [22]. LAB can be involved in the delivery of functional biomolecules and
ingredients into high quality gluten-free cereal products [23]. In the seafood industry, LAB are usually applied for product conservation, with the exception of traditional fish sauces in Southeast Asia. In recent years, novel fish products with various flavor and biochemical characteristics have been developed [24, 25].

Another direction of LAB application is beverage production. LAB are important components of the wine-making process: they are responsible for malolactic fermentation following alcoholic fermentation by yeast. Nearly all red wines and many white wines are obtained by these two fermentation steps. When all reducing sugars are converted to ethanol, yeast concentration declines and LAB start to grow consuming residual sugars and transforming numerous wine components. New aromas may improve wine bouquet, whereas those revealed during alcoholic fermentation by yeast are likely to vanish or change after malolactic fermentation. Some strains of LAB could even spoil wine during the process [26].

LAB are part of normal microflora of gastrointestinal and genitourinary tracts, hence they are used as components of probiotics. Beneficial effects of probiotics are provided by several mechanisms. Antagonistic action toward pathogenic bacteria may be manifested by decreasing the luminal pH through production of volatile short-chain fatty acids (SCFA), such as acetic, lactic or propionic acid; rendering specific nutrients not digestible by pathogens; decreasing the redox potential of the luminal environment; producing hydrogen peroxide under anaerobic conditions and specific inhibitory compounds such as bacteriocins affecting other bacteria [27, 28]. Besides the above-mentioned synthesis of various compounds, probiotics can be engaged in barrier function, modulation of the mucosal immune system, enhancement of food digestion and absorption and alteration of the intestinal microflora [29].

LAB can be used to control a wide range of diseases: diarrhea of various etiology [30], allergy [31–33], inflammatory bowel diseases [34] and hepatic diseases [35]. LAB are applied in the treatment of tumors such as colorectal cancer by several mechanisms: bacteria are able to cause apoptosis of tumor cells; they possess antioxidative activity; LAB stimulate immune response for cancer prevention and therapy; they are able to modify expression levels of selected genes and LAB suppress proliferation of cancer cells via synergistic action of adherence to tumor cells and production of SCFA [36]. Some LAB display cholesterol-lowering and antihypertensive effects and alleviate the symptoms of lactose intolerance in lactase-deficient individuals [37–40]. LAB were shown to promote immunomodulatory impact on human organism [41, 42].

LAB facilitate target delivery of valuable substances. Selenium is an essential trace element that protects organism from oxidative stress, helps maintain defense barrier against infections, modulates growth and development, provides for normal aging process, minimizes pregnancy complications and improves fertility and antiviral activity. Selenium-enriched probiotics have been shown to confer several health benefits on the host due to their antioxidative, antipathogenic, antimutagenic, anticancerogenic and anti-inflammatory activities [43].

LAB can be applied in prevention and treatment of animal diseases. Viruses, such as the infectious pancreatic necrosis virus and infectious hematopoietic necrosis virus, cause acute diseases of rainbow trout (Oncorhynchus mykiss) and several salmon species. The purified dextrans of
Lactobacillus sakei MN1 and Leuconostoc mesenteroides RTF10 have shown functional activity against these viruses [44]. In some cases, Enterococcus strains demonstrated prophylactic and therapeutic effect and stimulated immune response, growth and digestion in farm stock and pets [45]. Several studies testing the influence of various LAB species on pigs, poultry and ruminants established the elevated titer of beneficial bacteria and the reduction of potential pathogen load [46].

LAB and their products exhibit antifungal properties applicable in agriculture, food and wood industry. Fungi cause numerous diseases of crops and decrease yields. In addition, they impart an unpleasant smell, taste or appearance to feed and foodstuffs and produce a wide array of mycotoxins, making nutriment unsuitable for consumption. They cause adverse effects up to lethal cases after penetration into human or animal body [47]. LAB are able to inhibit fungal growth and to dispose of mycotoxins. The activity of LAB can be explained by synthesis of various compounds, competition for nutrients in the medium and/or acidification of the growth medium. Detoxification capacity can be related to adsorption of mycotoxins by the bacterial cell [48–50]. Even heat-killed cells of LAB may reduce toxin concentrations to safe levels in milk. Heat inactivation significantly enhanced aflatoxin M₁ removal by LAB [51]. Members of genera Lactococcus, Pediococcus, Leuconostoc and Lactobacillus are the most promising bacteria to inhibit fungal growth [50, 52, 53]. Both lactococci and yeast could delay or prevent the fungal deterioration of the baked food [53]. A multitude of studies showed LAB ability to block fungal spoilage of fresh fruits and vegetables, baked and dairy products and silage [54]. Besides, LAB were shown to inhibit the growth of wood-rotting fungi and subsequent wood decay [55].

1,3-Propanediol is a monomer in polymerization process producing polytrimethylene terephthalate, and it can also be used in the production of polyurethanes, polyesters and polyethers. A large number of microorganisms, including LAB, are capable of converting glycerol into 1,3-propanediol. The 1,3-propanediol concentration achieved in batch cultivation of Lactobacillus diolivorans equalled 41.7 g/L. This value could be increased to 84.5 g/L by co-feeding glucose and glycerol (in 0.1 molar ratio) and by adding vitamin B₁₂, the co-factor of glycerol dehydratases [56]. Recent studies have revealed possibility of applying LAB in biosurfactant production. Biosurfactants are a structurally diverse group of surface-active substances used in agriculture, food production, chemistry, cosmetics and pharmaceuticals [57].

LAB potential application area is bioremediation, e.g. treatment of wastewaters containing azo dyes. The latter make up the largest group of synthetic chemicals that are widely used in manufacturing of textile, leather, cosmetics, food and paper. During the industrial process, approximately 10–15% of the spent dye is discharged into wastewater. Azo pigments and their catabolic intermediates, like aromatic amines, distinguished by mutagenic and carcinogenic properties, obstruct light and oxygen transfer into water bodies, consequently affecting aquatic life. The research data indicate that the chemical can be catabolized and utilized by LAB strains and its degradation products are less toxic to growing Sorghum bicolor culture than the original azo pigment [58].

LAB and their products can be used for crude oil recovery. One third to a half of the world oil reserves are deposited in carbonate rock. They tend to have very low permeability that can be
improved by acid injection. Microbial acid producers, like LAB, may provide a solution for the problem. They are injected with nutrient substrate into the well where bacteria produce lactic acid reacting with CaCO$_3$. The water solubility of formed calcium lactate is approximately 80g/L as compared to 15mg/L for CaCO$_3$. Lactic acid may also be used for the removal of carbonate or iron scale from oilfield equipment [59].

4. Waste degradation and utilization by lactic acid bacteria

One-third of food intended for human consumption is lost or wasted globally at all steps from initial agricultural production to final household consumption. It amounts to about 1.3 billion tons per year [60]. Food wastes are mainly composed of carbohydrate polymers, such as starch, cellulose and hemicelluloses, plus lignin, proteins, lipids, organic acids and inorganic remainder. Total sugar and protein contents are in the range of 35.5–69 and 3.9–21.9%, respectively [61]. LAB may grow in any environment rich in carbohydrates, so that they can be found in various food products (milk, meat and vegetables), plants, as part of the normal human and animal microbiota. Food wastes are potential sources of nutrients for growth of LAB and production of valuable compounds.

Large volumes of waste generated by fishing, aquaculture or food processing are dumped into the sea without pretreatment. It causes grave environmental problems. This challenge can be met by introducing rich organic nutrients in the formulated optimum media for microbial cultivation. Enzymatic hydrolysate of octopus processing wastewater served as a good source for LAB growth (L. lactis and Pediococcus acidilactici) and synthesis of bacteriocins (nisin and pediocin, respectively). The maximal production of biomass and nisin by L. lactis was observed in the media with low concentration of enzyme papain and short time of hydrolysis (4 h). In case of pediocin, the highest production was attained in the media hydrolyzed with papain, trypsin and pepsin within 10 h period. Consequently, marine peptones are promising alternative nutrients in the media and their fermentation is a possible solution of wastewater problem [62].

Fish viscera waste can be used in preparation of silage intended as animal feed. Application of LAB makes bio-silage process simpler, faster, more environmentally friendly and cost-efficient than chemical technology. LAB strains produce metabolites and adjust pH values for bio-silage fermentation and preservation [63].

Brown juice, waste of the green crop drying industry, contains nutrients such as carbohydrates, organic acids, vitamins and minerals suitable for production of L-lysine. Pretreatment is required to convert brown juice into a stable, storable product that can be used for microbial fermentation. Traditional heat sterilization at 121°C for 20 min in batch procedure or at 140°C for a few seconds in continuous process inactivates valuable enzymes and consumes a lot of energy. When LAB deplete the constituent carbohydrates, the juice can be heat sterilized and used as a nutrient and water source for L-lysine production by Corynebacterium after addition of a carbon source and neutralization of the lactic acid by, e.g., ammonia. Alternatively, the lactic acid present in the medium can be utilized by Corynebacterium and converted to L-lysine [64].
LAB can be used for waste preservation. Fermentation of hatchery wastes, including infertile eggs, dead embryos, cull chicks and shells from hatched chicks, by bacteria *Pediococcus acidilactici* and *Lactobacillus plantarum* and products of *Streptococcus faecium* M74 exerted significant effects upon nutritional composition of the treated substrate. Additionally, LAB action reduces or eliminates pathogenic bacteria such as *Salmonella* species and *Escherichia coli*. These are important steps in recycling hatchery by-products into feed ingredients instead of landfilling waste [65]. Rations with fermented hatchery wastes showed no negative effect on broiler chicken. Their body weight gain and feed conversion at all stages were comparable to the control. In some cases, the parameters such as ready to cook carcass and wing yield significantly exceeded control values [66].

Lactic acid is the main product of LAB. The use of waste substrates for production of lactic acid by LAB is described in Section 6.

5. Food additives. Waste for the production of chitin and chitosan

Food additive is any substance added to food to improve its quality. These compounds are used in production, processing, treatment, packaging, transportation or storage of food. Food additives are applied to secure safety and freshness of products that could be spoiled by environment and microorganisms, to upgrade food nutritional value or modify taste, texture and appearance of consumable products. LAB are known to promote food quality and flavor from ancient times, but they also produce specific beneficial compounds that can be used for food supplementation or for extraction of valuable substances such as chitin.

Microbial contamination poses serious safety and quality problems in food industry. Bacteriocins are antimicrobial peptides produced by bacteria, which possess the ability to kill or inhibit other bacteria. The bacteriocins were first characterized in Gram-negative bacteria, but later they were observed in other bacterial groups, including LAB. These compounds are often confused with other antimicrobials or antibiotics. Unlike most antibiotics, which are secondary metabolites, bacteriocins are usually ribosomally synthesized and sensitive to proteases, whereas generally harmless to the human body and surrounding environment. Besides, bacteriocins have narrower spectrum of activity opposite to antibiotics. Bacteriocins are generally divided into several classes. Class I, or the lantibiotics, are small (<5 kDa) thermally stable peptides that contain lanthionine, methyl-lanthionine and dehydrated amino acids. Subclass Ia are linear structure peptides with membrane-disrupting mode of action, and subclass Ib are globular structure peptides with cellular enzymatic action. Class II containing small (<10 kDa) heat-stable, unmodified non-lanthionine membrane-active peptides is subdivided into five subclasses. Subclass IIa are pediocin-like *Listeria*-active peptides with a consensus amino acid sequence Tyr-Gly-Asp-Gly-Val-Xaa-Cys in the N-terminal position. Subclass IIb consists of two different unmodified peptides forming a fully active poration complex. Subclass IIc are circular peptides. Subclass IId are linear, non-pediocin-like, single-peptide bacteriocins and subclass IIe bacteriocins are non-ribosomal siderophore-type post-translation modification peptides with the serine-rich carboxy-terminal region. Class III bacteriocins are large molecular weight (>30 kDa), thermally
unstable proteins that can be further subdivided into two distinct groups with respect to cell lysis. Class IV forms large complexes with other macromolecules [67–69]. Due to sensitivity to proteases, bacteriocins are probably digested in the gastrointestinal tract into small peptides and amino acids. Since bacteriocin-producing bacteria are present in many types of food since ancient times, bacteriocins are considered as basically safe food additives [67]. The main perspective for these compounds is food preservation. There are many studies regarding the role of bacteriocins in conservation of dairy, meat, seafood and vegetable products [70–73]. However, only few bacteriocins are used as commercial biopreservatives. The most well-studied and used bacteriocin is nisin, first isolated from L. lactis ssp. lactis in 1928 [74]. Nisin approved as food additive in more than 50 countries, including USA and Europe, is marketed as Nisaplin®. It was included into the European food additive list under the number E234 with no recorded adverse effects. Nisin inhibits closely related species as well as food-borne pathogens such as Listeria monocytogenes and many other Gram-positive spoilage microorganisms [70]. Another commercially available bacteriocin is pediocin PA-1, marketed as Alta® 2341, which inhibits growth of L. monocytogenes [72].

Exopolysaccharides (EPS) of LAB are branched, repeating units of sugars or sugar derivatives produced extracellularly. They are involved in the protection of bacteria from adverse factors. EPS of LAB are versatile in molecular weight, linkages, solubility and degree of branching. The molecular mass of EPS ranges from 10 to 1000 kDa. Most LAB produce polysaccharides extracellularly from sucrose by glycansucrases or intracellularly by glycosyltransferases from sugar nucleotide precursors [75]. These compounds are widely applied in food industry as adjuvants, emulsifiers, carriers, stabilizers, sweeteners, bulking agents, extenders and so on. [76, 77]. EPS of LAB also find use in medicine. They prevent blood coagulation and facilitate blood flow, tissue transfer, tumor treatment, serve as lubricants, carriers, osmotic and hypocholesterolemic agents, etc. [77].

Low calorie sugars of LAB origin are recognized as vital ingredients in diabetic foodstuffs. Mannitol, sorbitol, xylitol, erythritol and D-tagatose are sweeteners produced by LAB. Mannitol is used as a sweet-tasting bodying and texturing agent. It retards sugar crystallization and is intended to increase the shelf life of foods. Crystalline mannitol exhibiting very low hygroscopicity is indispensable in products that keep stability at high humidity. The polyol is usually manufactured by high pressure hydrogenation of fructose/glucose mixtures; however, bacteria can also be used as sources of the compound. Lactobacillus intermedius B-3693 was shown to yield mannitol from fructose. For example, 0.70 g of mannitol per gram of fructose can be produced from 250 g/L fructose. It was established that one-third of fructose could be replaced by glucose, maltose, galactose, mannose, raffinose or starch with glucoamylase, or two-thirds of fructose could be replaced by sucrose for successful mannitol production [78]. D-tagatose can be used as a low-calorie sweetener. The sweetness profile of D-tagatose is similar to that of sucrose, but it is detected a bit sooner than that of sucrose. D-tagatose is catabolized via tagatose-6-phosphate pathway, a branch of galactose metabolism, by some microorganisms such as Lactobacillus casei and L. lactis. L-arabinose isomerase used in tagatose production was found in L. plantarum and Bifidobacterium longum [79]. Sorbitol is a low-calorie sugar alcohol widely used in food industry. This polyol has a relative sweetness of around 60% when compared to sucrose and displays 20 times higher solubility in water than mannitol. Sorbitol is applied as
sweetener, humectant, texturizer and softener in production of chewing gum, candies, desserts, ice cream and diabetic food. *L. plantarum* produces sorbitol with efficiency 61–65% from fructose-6-phosphate by reverting the sorbitol catabolic pathway in a mutant strain deficient for both L- and D-lactate dehydrogenase activities [80]. D-xylitol is a 5-carbon polyol used as a natural sweetener in food and confectionary industry and known for its anticariogenic properties. The recombinant strain *L. lactis* was able to produce D-xylitol during cometabolism of glucose and D-xylose. Xylitol synthesis reached productivity 2.72 g/L/h [81]. *Oenococcus oeni* has been reported to produce erythritol. This polyol is another compound that can be used as sugar substitute [82].

Antioxidant is the compound inhibiting oxidation of other molecules by free radicals. Although synthetic antioxidants are more effective, natural antioxidants are characterized by simpler structure, higher stability and safe immune response. Substances with potential antioxidant activity have been derived from many animal and plant sources. LAB products also show this kind of activity [83]. Some studies demonstrated LAB contribution in production of peptides showing antioxidant activity, with potential food and pharmaceutical applications [84, 85]. However, further investigations are required to evaluate prospects of peptides.

Vitamins are substances essential for metabolic processes. They regulate biochemical reactions in the cell. Some of them function as precursors of coenzymes. Humans are incapable of synthesizing most vitamins, so that they have to be provided from food or synthesized by gut microflora. Regrettably, vitamins are easily degraded during food processing or cooking. Certain strains of LAB possess the property to synthesize vitamins and hence can be engaged in elaboration of enriched fermented foods. Studies indicated LAB production of B-group vitamins and vitamin K [86, 87].

Conjugated linoleic acid (CLA) isomers are other compounds with important physiological properties. CLA represent the family of octadecadienoic acid (18:2) isomers, which have a pair of conjugated double bonds along the alkyl chain. There are 28 known CLA isomers. They are characterized by anticancer, antidiabetic, antiatherosclerotic and anti-osteoporosis activities, complemented by defattening and immune-stimulating functions. The use of LAB and *Bifidobacteria* allows to increase CLA content of fermented dairy products, with no adverse effects described to date. Attempts to raise CLA productivity of LAB have been reported [88].

Apart from nutrient balance, a key food characteristic is flavor. Consumers need not only healthy but also delicious food. LAB showed ability to degrade phenolic acids generating compounds responsible for aroma. Phenolic compounds are directly related to sensory food characteristics such as flavor, astringency and color. In addition, they show antioxidant activity [89]. LAB metabolize phenolic acids by decarboxylation and/or reduction. The products of phenolic acid decarboxylase action are vinylcatechol, vinylphenol, vinylguaiaicol, pyrogallol and catechol; reduction of hydroxycinnamic acids yields dihydrocaffeic and dihydroferulic acids [90–92]. Strains with high enzymatic activities can be used to enhance the flavor of cheeses [93]. The volatile flavor components, which predominantly determine the typical odor of cheese, are subsequently derived from the activity of amino acid converting enzymes [94].
Chitin is a polysaccharide composed of N-acetyl-D-glucosamine units. It is the second most abundant biopolymer on Earth after cellulose and it is a structural component of the arthropod exoskeleton and of the cell walls of algae, fungi and yeast. Chitin is the source of chitosan, polysaccharide with numerous applications in the area of food and nutrition, in agriculture and environmental protection, medical, dietetic and cosmetic products. Chitin is widely used to immobilize enzymes and whole cells further engaged in clarification of fruit juices and processing of milk [95]. Chitosan and its derivatives can be applied as thickeners and stabilizers for sauces, fungistatic and antibacterial coating for fruit, preservatives, dietary fibers and cholesterol reducers [96]. Chitin and chitosan are non-toxic compounds displaying excellent biological properties such as biodegradation in the human body, immunological, antibacterial and wound-healing activity [97–100]. They also possess chelating ability and adsorption capacity and promote disposal of unwanted substances or extraction of valuable compounds [101]. Derivatives such as chitosan-sugar complexes show the potential to act as better antimicrobial and antioxidant agents than chitosan itself. Antimicrobial activity of chitosan is displayed by several mechanisms. The available amino group in chitosan structure provides for absorption of the nutrients necessary for bacterial growth. Interaction between the positive charge of chitosan molecule and the negative charge of microbial cell membrane changes membrane permeability. Chitosan film formation over the surface of microbial cell membrane prevents the nutrients from getting into the cell [102].

Chitin is associated with proteins, lipids, pigments and mineral deposits. Therefore, chitinous materials have to be pretreated to remove by-components. Chitin can be extracted by various ways, including LAB introduction. However, demineralization and deproteinization of the chitinous material depend primarily on fermentation conditions. Ninety-one percent of deproteinization with lower level of demineralization can be reached under optimal conditions by L. helveticus using date juice as an alternative to glucose that decreased the degree of deproteinization to 76% [103]. The other strain Pediococcus acidolactici CFR2182 carried out efficient fermentation of shrimp waste resulting in 97.9% deproteinization and 72.5% demineralization [104]. The epiphytic L. acidophilus SW01 culture isolated from shrimp waste quickly removed minerals and proteins from that substrate to residual 0.73 and 7.8% values, respectively, after 48 h fermentation. In the pilot scale fermentation, the mineral and protein contents fell to 0.98 and 8.44%, respectively, after 48 h fermentation [105]. The combination of lactic acid bacteria (Lactobacillus paracasei) and protease-producing bacteria (Serratia marcescens) can also be effective for extraction of chitin. LAB intensely dissolved mineral CaCO3 by producing organic acid and S. marcescens degraded proteins by producing extracellular proteases. The extent of demineralization reached the highest mark of 97.2%, but the percentage of deproteinization in cofermentation was 52.6% on day 7 due to unfavorably low pH for proteolytic activity [106]. Mixed cultures of L. lactis and Teredinobacter turnirae displayed splendid activity in mineral and protein removal, respectively, and promoted chitin extraction, especially when T. turnirae was first inoculated [107]. LAB can recover chitin with accessory compounds such as pigment astaxanthin reported to be an excellent antioxidant and anticarcinogenic substance [108]. Microbial method is more effective for isolation of chitin when compared with chemical method. Adding Fe (NO3)3 as extra nitrogen source increases...
yield twice. Organic acids, like lactic acid, can be produced at low cost by bacteria, are less harmful to the environment and can preserve characteristics of the purified chitin, whereas the organic salts from demineralization process can be used as environmentally friendly deicing agents or as preservatives [109].

6. Lactic acid: use of waste substrates for production of lactic acid by LAB

Lactic acid, or 2-hydroxypropanoic acid, is water soluble and highly hygroscopic organic acid with ubiquitous distribution in nature. Lactic acid was discovered in 1780 by C.W. Scheele in sour milk, and in 1881 Fermi obtained this compound by fermentation, resulting in its industrial production. Lactic acid is widely used in food, pharmaceutical, cosmetic and other manufacturing sectors. In the chemical industry, lactic acid is treated as a raw material for production of lactate ester, propylene glycol, 2,3-pentanedione, propanoic acid, acrylic acid, acetaldehyde and dilactide. This compound can even be used for fabrication of polylactic acid (PLA), sustainable bioplastic material mainly applied in packaging. Lactic acid functions as a descaling agent, pH regulator, neutralizer, chiral intermediate, solvent, humectant, cleaning aid, slow acid-release, metal complexing and antimicrobial agents. Technical-grade lactic acid is used in leather tanning industry as an acidulant for deliming hides. Besides moisturizing and pH adjusting effect, the substance is characterized by antimicrobial activity, skin lightening and hydrating action in cosmetic industry. In medicine, lactic acid is applied in tabletting, prostheses, surgical sutures, controlled drug delivery systems and electrolyte solutions [110]. However, food industry is the main consumer of lactic acid. Food and food-related applications account for approximately 85% of lactic acid demand, whereas the other industrial sectors cover the remaining 15% [111]. Lactic acid and its salts are used as antimicrobials, flavor enhancers, stabilizers, thickeners, humectants, emulsifiers, firming and leavening agents and so on [110, 112]. Lactic acid is applied in a wide variety of foodstuffs, such as candies, bread and bakery products, soft drinks, soups, sherbets, dairy products, beer, jams and jellies, mayonnaise and processed eggs [113].

The global lactic acid demand estimated to be 714.2 kilo tons in 2013 is expected to reach 1960.1 kilo tons by 2020 [114]. Substrates for lactic acid production should be characterized by cheapness, low contamination level, year-round availability, rapid fermentation rate and high yields of lactic acid from fermentation.

Food waste has high starch content and is rich in nutrients, including lipids and proteins, and therefore it represents a potential renewable resource for lactic acid production. Additionally, protease, temperature and CaCO₃ cause significant linear effects on production, whereas α-amylase and yeast extract show minor effects. Under the optimal conditions, L. planta-rum produced maximum amount of lactic acid from dining hall food waste [115]. Municipal organic solid waste (MOSW) is the discharge consisting of kitchen and garden residues. MOSW possesses high energy and nutritional value for lactic acid production. Lactic acid productivity after 24 h was 0.79 ± 0.05 g/L/h in fermenters with pH 5.0 and 0.71 ± 0.05 g/L/h in fermenters with uncontrolled pH [116].
Sugarcane juice containing 13–16% sucrose is renewable, abundant and cheap source of carbon for lactic acid production. *Lactobacillus* sp. strain FCP2 grown on sugarcane juice for 5 days produced 104 g/L lactic acid with 90% yield. Higher yield (96%) and productivity (2.8 g/L/h) were obtained when the strain was cultured on 3% w/v sugarcane juice for 10 h. Addition of cheap nitrogen sources such as silk worm larvae, beer yeast autolysate and shrimp waste led to increase in lactic acid production over that attained with yeast extract [117]. Molasses is the by-product of refining sugarcane or sugar beet. It contains sucrose (31%), glucose (9.5%), fructose (10%), nitrogen (0.95%) and may be used as cheap and available medium for production of various compounds, including lactic acid. *L. delbrueckii* mutant Uc-3 in batch fermentation process produced 166 g/L lactic acid from 400 g/L molasses [118]. Lactic acid concentration 134.9 g/L was recorded at molasses concentration 333 g/L using *Enterococcus faecalis* culture [119]. Glycerol is the main by-product of biodiesel industry and it can be utilized as a carbon source to yield organic acids, e.g. lactic acid. Strain *Lactobacillus* sp. CYP4 produced 39.41 mM lactic acid with conversion percentage 39.27% [120]. Liquid waste from potato processing industry (chips manufacturing) can be used as substrate for lactic acid production. Waste with MRS medium (lacking peptone, yeast extract and glucose, but containing malt extract, galactose and manganese sulfate) in 4:1 ratio provided for 16.09 g/L concentration of lactic acid by *L. casei* culture [121].

Brewers’ spent grain (BSG) represents the major by-product of brewing industry accounting for about 85% of total residues left after the mashing and lautering processes and it is available in large amounts all year around [122]. Chemical composition of BSG varies depending on the barley variety, the harvest time, malting and mashing conditions; however, its hydrolysates are suitable substrates for lactic acid production. Generation of the desired metabolite through fermentation of hydrolysate resulting from BSG pretreatment with aqueous ammonia was 96% higher than that following acid-alkaline treatment and constituted 17.49 g/L. The maximum value was obtained after addition of nitrogen source (yeast extract) to aqueous ammonia-treated BSG (22.16 g/L) [123]. Additional use of inverterase from grape juice for sucrose hydrolysis of canned pineapple syrup, a food processing waste, resulted in lactic acid concentrations 20 and 92 g/L generated by *L. lactis* from 20 and 100 g total sugars/L [124].

About 30% of annual global cheese whey production remains underutilized, ending up as waste or animal feed [125]. Besides, most dairy manufactures do not have proper treatment systems for whey disposal. The main components of whey are lactose (approximately 70–72% of the total solids), whey proteins (approximately 8–10%) and minerals (approximately 12–15%) utilized by LAB with lactic acid production. Various studies with free and immobilized cells proved efficiency of LAB application [126]. Scotta is the main by-product of ricotta cheese production containing proteins (0.15–0.22%), salts (1.0–1.13%) and lactose (4.8–5.0%). Scotta may be considered as a source of lactose and other nutrients with potential biotechnological applications such as lactic acid production. The addition of nutritional supplements to medium with scotta led to lactic acid productivity about 2 g/L/h. The use of mixed cultures reduces the need for nutrient supply, with no detrimental effects on the production parameters as compared to pure cultures [127]. Mussel processing waste, liquid by-product of industrial steam treatment
of mussels, contains glycogen as the main component that can be utilized by LAB with protein and phosphorus supply and pH control [128].

Deficiency of the nitrogen source usually decreases yield of lactic acid. Moreover, nitrogen source is the most expensive component of microbial growth media. Ram horn hydrolysate (RHH) was shown to be rich in both organic and inorganic compounds and hence considered as an excellent source of nitrogen and minerals in fermentation medium because of its amino acid and mineral contents. The optimal concentration of RHH for production of lactic acid was 6%. Concentrations higher than 6% had an inhibitory effect due to high amounts of heavy metals. 44 g/L concentration of lactic acid was generated on medium with RHH by 26 h of fermentation with nearly 100% sugar consumption in contrast to control medium (36 g/L and the degree of sugar consumption 82%) [129].

Experiments with production of lactic acid were performed on pineapple juice waste [130], waste potato starch [131], cassava powder [132], waste banana [133], kitchen waste [134] and fish waste [135].

Lignocellulosic hydrolysates also can be used for lactic acid production. Lignocellulosic biomass, organic material of biological origin, represents the most abundant global source of unutilized biomass. Lignocellulosics are typically composed of cellulose (insoluble fibers of β-1,4-glucan), hemicellulose (noncellulosic polysaccharides, such as xylans, mannans and glucans) and lignin (a complex polyphenolic structure) with lesser amounts of minerals, oils and other components. The proportion of biomass constituents varies among species. LAB are not able to digest these components, therefore, pretreatment and enzymatic hydrolysis stages are essential [136]. For example, dilute acid pretreatment efficiently hydrolyzes hemicellulose to xylose, arabinose and glucose and thereby enables further enzymatic digestion of cellulose to glucose. The obtained compounds are utilized by LAB. However, substances toxic to fermentative organisms such as furfural, phenolic derivatives and inorganic acids are also produced during the pretreatment process. Strains S3F4 \( (L. \text{ brevis}) \) and XS1T3-4 \( (Lactobacillus \text{ plantarum}) \) exhibited the ability to utilize various sugars present in dilute-acid hydrolysates of corn stover and corncobs, especially S3F4 converting hydrolysates into lactic acid without detoxification. The strain showed strong resistance to the potential inhibitors, furfural, and ferulic acid. The maximum lactic acid concentration achieved by S3F4 fermentation was 39.1 g/L from corncob hydrolysate [137].

The food processing industry generates significant amounts of solid wastes. For example, over 50% of the orange fruit is transformed into peel waste during the juice making process [138]. Food processing wastes are usually utilized via cattle feeding, burning and landfills, but they contain significant amounts of carbohydrates, proteins and lipids that could be used to produce valuable compounds such as lactic acid. Research with different agricultural (orange, banana and potato) peel wastes fermented by mixed cultures showed that lactic acid was the predominant chemical produced in all fermentation broths. The abundance of LAB rapidly increased during fermentation and genus \( Lactobacillus \) dominated at the end of process [139]. LAB, mainly \( Lactobacillus \) species, successfully produced lactic acid from other lignocellulosic substrates (Table 1).
7. Use of LAB in production of bioenergy and biogas

The latest decades have witnessed growing interest in production of green energy. Fossil fuels adversely influence the environment owing to emission of carbon dioxide, triggering search for inexpensive renewable sources of energy that do not affect the surrounding nature. Microbial fuel cells (MFC) are devices that utilize organic and inorganic wastes and transform their chemical energy into electrical energy. MFC consist of anode and cathode chambers, physically separated by a proton exchange membrane (PEM). Microorganism in the anode section oxidizes the organic substrates and produces electrons and protons. The protons are conducted to the cathode chamber through PEM, and the electrons are conveyed via external circuit. Protons and electrons are reacting in the cathode chamber along with parallel reduction of oxygen to water. A steady current is generated by this process within the wire connecting anode and cathode. Besides generation of bioelectricity, MFC additionally resolve problem concerning utilization of waste [154].

MFC research has been conducted during several decades, but studies engaging LAB for generation of bioenergy were initiated only in recent years. Fe(III)-reducing bacterium

<table>
<thead>
<tr>
<th>Lignocellulosic substrates</th>
<th>Bacteria</th>
<th>Lactic acid production</th>
<th>References</th>
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<tbody>
<tr>
<td>Alfalfa fiber</td>
<td>Lactobacillus delbrueckii</td>
<td>0.606 g/g</td>
<td>[140]</td>
</tr>
<tr>
<td></td>
<td>Lactobacillus pentosus</td>
<td>0.59 g/g</td>
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<tr>
<td>Apple pomace</td>
<td>Lactobacillus rhamnosus</td>
<td>32.5 g/L</td>
<td>[141]</td>
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<tr>
<td>Cellulosic biosludges</td>
<td>Lactobacillus rhamnosus</td>
<td>39.4 g/L</td>
<td>[142]</td>
</tr>
<tr>
<td>Chips of oak wood</td>
<td>Enterococcus faecalis</td>
<td>24-93 g/L</td>
<td>[143]</td>
</tr>
<tr>
<td>Milled newspaper</td>
<td>Lactobacillus delbrueckii</td>
<td>24 g/L</td>
<td>[144]</td>
</tr>
<tr>
<td>Municipal solid waste</td>
<td>Lactobacillus pentosus</td>
<td>65 g/L</td>
<td>[145]</td>
</tr>
<tr>
<td>Pine needles</td>
<td>Co-culture of Lactobacillus delbrueckii and Lactobacillus pentosus</td>
<td>45.10 g/L</td>
<td>[146]</td>
</tr>
<tr>
<td>Recycled paper sludge</td>
<td>Lactobacillus rhamnosus</td>
<td>73 g/L</td>
<td>[147]</td>
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<tr>
<td>Sugarcane bagasse</td>
<td>Lactococcus lactis</td>
<td>10.85 g/L</td>
<td>[148]</td>
</tr>
<tr>
<td>Turmeric residue</td>
<td>Lactobacillus paracasei</td>
<td>97.13 g/L</td>
<td>[149]</td>
</tr>
<tr>
<td>Vine-trimming wastes</td>
<td>Lactobacillus pentosus</td>
<td>21.8 g/L</td>
<td>[150]</td>
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<tr>
<td>Waste cardboard</td>
<td>Lactobacillus corporeformis</td>
<td>0.514 g/g</td>
<td>[151]</td>
</tr>
<tr>
<td>Wheat straw</td>
<td>Lactobacillus pentosus</td>
<td>6.6–6.7 g/L</td>
<td>[152]</td>
</tr>
<tr>
<td></td>
<td>Lactobacillus brevis</td>
<td>4–4.7 g/L</td>
<td></td>
</tr>
<tr>
<td>Wood chips of Eucalyptus globulus</td>
<td>Lactobacillus delbrueckii</td>
<td>48–62 g/L</td>
<td>[153]</td>
</tr>
</tbody>
</table>

Table 1. Lignocellulosic substrates in lactic acid production.
**Enterococcus gallinarum** MG25 turned out to be electrochemically active strain. It appears that MG25 can transfer electrons to the electrode as electron acceptor, so that the strain is expected to have promising MFC application prospects [155]. *L. lactis* is normally homolactic bacterium under anaerobic conditions. It lacks the genes that encode biosynthesis of heme. When a source of heme is provided, the respiratory chain is activated and the bacterium can oxidize NADH using O₂ as terminal electron acceptor. If lower potential terminal electron acceptors are engaged, such as hexacyanoferrate, ferric citrate or cupric chloride, the electron transfer chain is not required in its entirety up to cytochrome oxidase step, with final electron transmission carried out mostly by quinones. *L. lactis* was observed to perform extracellular electron transfer to anodes by utilizing at least two soluble redox mediators (one of these two mediators was 2-amino-3-dicarboxy-1,4-naphthoquinone) with acetate and pyruvate production and electricity generation [156]. Mixed cultures also can be used in MFC. While *Shewanella oneidensis* or *L. lactis* alone cannot generate electric current from glucose, they can do so in coculture. *L. lactis* converts glucose into lactate, which serves as electron donor to *S. oneidensis* [157]. *Lactobacillus bulgaricus* was tested as producer of electricity. The maximum power (201.8 mW/m²) was generated at optical density 0.5 by connecting in series MFC reactors with potassium permanganate as the electrolyte solution [158]. Further on, electricity output reached power density 393.23 mW/m² with LAB application [159]. Indium tin oxide (ITO) conductive glass anode modified by chitosan (CS) and α-Fe₂O₃ nanoparticles using LAB as the source of electrons raised considerably electricity generation. The maximum power density values of ITO blank, ITO/(CS/α-Fe₂O₃)/CS and ITO/(CS/α-Fe₂O₃)/CS were 0.035, 0.124 and 0.084W/m², respectively. The higher roughness of ITO/(CS/α-Fe₂O₃)/CS resulted in higher specific surface area available for growth of bacteria [160]. Following the trend, further development of MFC engaging LAB can be expected. Noteworthy, wastes are often applied in this technology, resolving thereby waste utilization problem.

Microbial electrolysis cell (MEC) is a technology similar to MFC, but this system recovers energy from substrates as valuable chemical compounds, like hydrogen. The latter is formed by reduction of protons with the transferred electrons in MEC. A microbial consortium demonstrated the ability to consume cheese whey as the sole carbon source yielding electricity or hydrogen. Cheese whey was fermented mainly by lactic acid bacteria (*Enterococcus* genus) and exoelectrogenic activity was expressed by *Geobacter* sp., utilizing acetate derived from fermentation as electron donor. The coulombic efficiency was 49.8% in the MFC system. In the MEC, hydrogen production reached 0.8 LH₂/L REACTOR/d and it proved the potentiality of cheese whey to be a good carbon source for bioenergy production [161].

Added to MEC and MFC, LAB may be involved in the production of biofuels such as hydrogen, methane (biogas), ethanol and butanol. Hydrogen is one of the most attractive energy carriers alternative to conventional fossil fuels. It does not affect environment and produces only water vapor and heat energy as the result of its burning. Hydrogen is a highly efficient energy source; its specific energy value equals 33 Wh/g. For comparison, the specific energy of methane is 14.2 Wh/g and coal is 9.1 Wh/g. The biological processes leading to hydrogen production are dark fermentation, photofermentation, direct and indirect biophotolysis, as well as anaerobic respiration of sulfate-reducing bacteria under conditions of sulfate depletion [162]. LAB are unable to produce hydrogen themselves, but can influence hydrogen gen-
eration by increasing or decreasing its production. LAB can act as seeds for self-floculated granule formation in hydrogen generation [163]. Another research revealed relation between the number of LAB and hydrogen production from simulated cheese processing wastewater via anaerobic fermentation using mixed microbial communities. More than 50% of the bacteria were *Lactobacillus* and about 5% of the isolates were hydrogen-producing *Clostridia* species. When H\textsubscript{2} production in the bioreactors decreased, concurrent reduction in the cell titer of genus *Lactobacillus* was also observed. It can be connected with pH important for H\textsubscript{2} production [164]. *Leuconostocaceae* were one of the predominant microbes in hydrogen-producing consortia in other experiment. Their role in the process is discussed [165]. When mixed cultures were used, *Lactobacillus amylovorus* utilized algal starch for lactic acid production and *Rhodobium marinum* produced hydrogen in the presence of light using lactic acid as an electron donor [166]. Products of LAB such as lactic acid also showed positive effect on hydrogen production. The addition of lactic acid to starch-containing medium could improve the hydrogen production rate and hydrogen production yield from 4.31 to 8.23 mL/h and from 5.70 to 9.08 mmol H\textsubscript{2}/g starch, respectively. The authors guessed that enhanced hydrogen production was associated with a shift from acetic acid and ethanol formation to synthesis of butyric acid as the predominant metabolite. The increase in hydrogen yield was attributed to the increase in the available residual NADH for H\textsubscript{2} production. However, when lactic acid was used as the sole carbon source, no significant hydrogen generation was observed [167]. *Clostridium diolis* JPCC H-3 on medium with acetic acid and lactic acid produced 2.85 mL H\textsubscript{2}/5 mL solution as compared to the control (0.63/5 mL solution) [168]. *Rhodobacter sphaeroides* GL-1 immobilized on polyurethane foam in a continuous flow bioreactor converted lactic acid to H\textsubscript{2} with an efficiency of 86% [169]. The hydrogen yield of *R. sphaeroides* RV was found to depend on lactic acid concentration, and maximum bacterial activity was observed at 100 mM influent lactic acid [170]. Nevertheless, other studies showed negative influence of LAB on hydrogen production. *L. paracasei*, *Enterococcus durans* and their supernatants inhibited hydrogen production via excretion of bacteriocins which have a deleterious effect on other bacteria. The inhibition of hydrogen production can be reduced by heat treatment for 30 min at temperatures ranging from 50 to 90°C and partially removed in the presence of protease trypsin inactivating bacteriocins [171]. The bacteriocin-producing LAB (mostly *Lactobacillus* spp.) were found to suppress hydrogen production during fermentation of cheese whey wastewater. At the same time, the highest H\textsubscript{2} yields were obtained when growth of *Lactococcus* spp. was associated with *Leuconostoe pseudomesenteroides*, although *Lactococcus* spp. is not recognized as hydrogen-producing strain [172]. Competition for resources between bacteria also reduces hydrogen production [173–175].

Biogas is a renewable energy source, which can be used as gaseous vehicle fuel and replace natural gas as a feedstock for producing chemicals and materials. Concerning biogas production, LAB are not directly involved in its generation, but the bacteria are able to influence methane yield. Crop characteristics, process parameters and management measures have a major impact on biogas yield. Ensiling with inoculated LAB is an appropriate method of storing feedstock for biogas production. Ensiling, prolonged storage and biological silage additives showed positive effects on methane yield of up to 11%. These could be attributed to increase in ratio of organic acids and alcohols. Changes in composition of fermentation products during ensiling and storage duration compensate for silage losses. Silage additives
either accelerate the ensiling process or stabilize the silage [176, 177]. Different crops showed various need in ensiling promoters. Additive-free ensiling resulted in minor losses (0–13%) in the methane potential of sugar beet tops, but more substantial losses (17–39%) in the methane potential of grass. Ensiling with supplements improved the methane potential of both substrates by 19–22% [178]. High concentrations of ethanol and butyric acid following clostridial and heterofermentative lactic acid bacterial fermentations were also accompanied by elevated specific CH₄ yield from grass [179]. The methane yield of maize silage treated with heterofermentative LAB was measured higher than from the corresponding solid residue, while the treatment of amaranth showed a significant decrease in methane yield from silage in contrast to solid residue [180]. Other studies showed that LAB failed to raise methane yield or had little effect [181–183]. One experiment indicated that silage from maize straw not exposed to ensiling preparations was characterized by the highest biogas yield [184]. LAB could not stimulate total methane production, but they were able to promote the methane production rate at the beginning of the process [183]. The other products, like food waste, could also serve as methane sources. However, lactic acid pre-fermentation of food waste caused acid inhibition of the methanogenesis. Methane yield was a bit higher compared to the control, but significantly lower when ethanol pre-fermentation was used [185]. Lactic acid exerted extremely negative influence on methanogenesis of kitchen waste [186]. Although application of LAB in the ensiling process does not always increase methane yield, these bacteria conduct preservation of silage used in biogas production. LAB lead to PH drop by producing organic acids (mainly lactic and acetic acids) and decrease risk of microbial contamination [187].

Ethanol is another renewable energy source derived from plant biomass. Global production of ethanol increased from 17.25 billion L in 2000 to over 46 billion L in 2007 [188]. Yeasts are one of the main producers of ethanol. Nevertheless, ethanol generation process may be influenced by several factors, including microbial contamination. LAB are very abundant in the process because of their tolerance to ethanol, low pH and high temperature. Some strains are able to grow in media with 16% ethanol [189]. Diverse species of LAB can be found in the bioethanol process [190, 191]. It was shown that lactic acid may affect yeast viability [192]. However, due to the above-mentioned features and ability to produce ethanol (heterofermentative pathway), LAB can also be considered as biofuel sources. L. buchneri NRRL B-30929 ferments solely glucose at pH 4.0 into lactate and ethanol at molar ratio 1.03:1. Equimolar amounts of ethanol and lactate are produced when only xylose is available for the strain [193]. Recombinant strain L. plantarum containing several genes of Sarcina ventriculi produced slightly more ethanol (90–130 mM) than the control [194].

Biobutanol is another promising fuel. Compared to ethanol, butanol is distinguished by higher energy content, higher octane number, lower latent heat, lower solubility in water, higher vapor pressure and inferior corrosive capacity. Additionally, butanol can be directly included in the current design of internal combustion engines. The species Clostridia are the natural producers of butanol. However, they are difficult to culture and butanol is characterized by toxicity to bacteria at concentrations over 20 g/L, far below its solubility in water (~70 g/L) [195]. As a consequence, other microorganisms are screened for butanol production. Due to high degree of alcohol tolerance, LAB became objects for genetic manipulations to select butanol-producing strains. The recombinant L. brevis strain containing the clostridial genes crt, bcd, etfB, etfA and hbd was able to synthesize up to 300 mg/L butanol comparable to recombinant E. coli (580 and 552 mg/L) and
*Pseudomonas putida* (120 mg/L) cultures [196–198]. Recombinant strains of *L. lactis* and *L. buchneri* containing clostridial thiolase produced about 28 and 66 mg/L butanol, respectively [199]. Some *L. brevis* strains were found to produce 2-butanol without recombination. These strains converted meso-2,3-butanediol to 2-butanol in a synthetic medium, but none of them showed the same ability in a complex medium such as MRS. It appears that the process is inhibited by some kind of repression mechanism [200].

LAB effects on energy generation are controversial. It was shown that LAB can be used for energy generation in MFC and MEC and production of butanol. However, influence of the bacteria on hydrogen, biogas and ethanol processes is complicated. LAB fail to generate hydrogen and biogas, but they and their products are able to increase or decrease the output of biofuels. Concerning ethanol, LAB may reduce yeast product yields or act as substrate providers. Contradictory impact of LAB on bioenergy generation requires further research to minimize negative effects and gain maximum benefits.

8. Use of LAB in food industry

Fermentation is the important process for manufacturing of products with desirable biochemical characteristics with the aid of microorganisms or enzymes. Fermentation plays at least five roles:

1. Enrichment of the diet through development of a diversity of flavors, aromas and textures in food substrates.
2. Preservation of food via lactic acid, ethanol, acetic acid and alkaline fermentations.
3. Biological upgrading of food substrates with proteins, essential amino acids, fatty acids and vitamins.
4. Detoxification in the course of food fermentation.
5. Saving cooking time and fuel requirements [201].

LAB from ancient times have been used in production of traditional foodstuffs. LAB are important microorganisms involved in manufacturing various dairy products such as yogurt, kefir, cheese, butter and so on. The latter account for about 20% of the global output of fermented products [202]. LAB can be divided into two groups depending on optimal growth temperature: mesophilic (20–30°C) and thermophilic (30–45°C). The flavor, texture and consistency may vary considerably when mesophilic or thermophilic cultures are used. Dairy industry mainly consumes starter cultures selected and maintained by subcultivation in milk. Several steps are carried out to obtain the required products [203, 204]:

1. Selection of starter cultures, optimization of medium and cultural conditions. These factors affect the yield of the product and its characteristics.
2. Pretreatment. This step includes various processes such as clarification, fat separation, standardization, evaporation, de-aeration, homogenization, pasteurization and so on. Pretreatment aim is to adjust dairy substrate characteristics and eliminate microorganisms able to interfere with fermentation process. The milk is then cooled to the appropriate fermentation temperature.

3. Fermentation. After pretreatment step, starter cultures are added and incubated at optimal temperature for the definite period. The bacteria ingest the lactose and release some compounds, like lactic acid. Production of lactic acid results in increased acidity causing milk proteins to denature and aggregate and growth inhibition of other acid-sensitive species.

4. Postfermentation step. After the end of fermentation process, the product may be subjected to downstream processing and upgrading (addition of flavorings, homogenization, filtration, etc.).

5. Packing, labeling, storage and market distribution of the product.

Manufacturing of fermented meat, soy, vegetables and baking products using LAB is carried out by a similar scheme. LAB provide the characteristic flavor and produce acids (e.g. lactic acid) that lower pH of the products and inhibit growth of spoilage microorganisms [205].

As mentioned in Section 5, LAB are sources of various compounds that can be used as food additives. Studies showed high efficiency of LAB in product enrichment with these additives. *L. amylovorus* CRL887 was able to produce significant concentrations of folate, or vitamin B9 (81.2 ± 5.4 μg/L), on folate-free cultural medium. Co-fermentation with B9 producing starter cultures *L. bulgaricus* CRL871 and *Streptococcus thermophilus* CRL803 and CRL415 yielded yogurt with high folate concentration (263.1 ± 2.4 μg/L). A single portion of the product provides for 15% of the recommended dietary allowance [206]. *L. plantarum* was shown to increase about twofold and threefold riboflavin (vitamin B2) content in pasta and bread, respectively [207]. *L. reuteri* CRL1098 from sourdough was able to produce vitamin B12 or cobalamin [208]. *L. lactis* ssp. *cremoris* YIT 2012 and *Leuconostoc lactis* YIT 3001 produced 9–123 μg of vitamin K2/L in defatted dry milk and soymilk medium, respectively, providing beneficial property for dietary supplement [209].

Concerning bacteriocins, nisin has been approved worldwide to use as a natural food preservative in food industry. It demonstrated a long record of food preservation efficiency [210]. Other bacteriocins also have practical applications. Paracin C produced by *L. paracasei* CICC 20241 induced extensive cell damage and disintegration of *Alicyclobacillus acidoterrestris* causing spoilage of fruit juices. The bacteriocin additionally reduced thermal resistance of bacterial spores [211]. *L. paracasei* subsp. *tolerans* from kefir produced bacteriocin inhibiting both fungi and bacteria [212]. Bacteriocin produced by *P. acidilactici* showed suppressing and bactericidal effect on *L. monocytogenes* in meat products [213]. *Lactobacillus* species isolated from different fermented cereal gruels demonstrated inhibitory action on growth of various target organisms [214]. Bacteriocin of *Enterococcus faecium* CN-25 isolated from fermented fish product completely inhibited growth of *L. monocytogenes* at the minimum concentration 2.38 mg/mL [215].
CLA-producing strains may be used in the food industry to derive products with increased CLA content. Strains of the genera *Bifidobacterium*, *Lactobacillus* and *Lactococcus* are able to enrich skim milk with CLA (in the range of 40–50 μg CLA/mL) [216]. Administration of *Lactobacillus* strains led to significant increase in CLA concentrations 0.2–1.2 mg/g fat in eggs and 0.3–1.88 mg/g fat in broiler chicken cuts [217]. *L. plantarum* from fermented pickle brines exhibited high CLA-producing ability in the presence of linoleic acid [218].

EPS of LAB have a wide application range. They can be used to modify certain food features. Incorporation of EPS may provide viscosity, stability and water-binding functions that may contribute positively to the odor, texture and taste of fermented dairy products [219]. *S. thermophilus* zw TM11 induced high exopolysaccharide content (380 mg/L) and viscosity (7716 mPa/s) of fermented milk. The co-culture of this strain with *L. delbrueckii* subsp. *bulgaricus* 3.4.5 caused low syneresis (8.5%), better texture and sensory perception of fermented yogurt [220]. EPS from *S. thermophilus* MR-1C significantly increased moisture retention in low-fat mozzarella. The cheese with low moisture content has a tough and rubbery texture and requires more heat for melting [221]. EPS-producing LAB were used in the production of Swedish ropy milk with proper level of viscosity [222]. Sour cream fermented by *S. thermophilus* strains producing capsular exopolysaccharides was characterized by low syneresis, high apparent viscosity and increased adhesiveness and gumminess [223]. EPS-producing strains of *S. thermophilus* showed reduced freezing mortality when LAB were introduced into frozen dairy desserts as a source of β-galactosidase hydrolyzing lactose and producing the absorbable monosaccharides glucose and galactose [224]. Besides dairy industry, EPS are used in bakery. *Weissella cibaria* WC4 and *L. plantarum* LP9 were able to produce EPS that increased the viscosity of baked product and the resulting bread was distinguished by higher specific volume and lower firmness [225]. EPS can improve not only taste, structure, consistency and shelf life of food products but also probiotic characteristics. Fermented milk with EPS-producing *S. thermophilus* culture and purified EPS resuspended in milk were effective for gastritis prevention [226]. Three strains of *L. delbrueckii* subsp. *bulgaricus* isolated from home-made yogurt produced high amounts of EPS and showed cholesterol lowering effects [227].

Reactive oxygen species and free radicals take part in the development of degenerative diseases such as cancer, atherosclerosis and diabetes [228]. Foods containing antioxidative materials may be applied for prevention of these diseases. LAB demonstrated antioxidant activity and could be used in the production of food with required properties. The radical-scavenging activity of water/salt-soluble extracts from sourdough fermented by pool of LAB was significantly higher than in control chemically acidified dough. The highest activity was found for whole wheat, spelt, rye and kamut sourdoughs [85]. It was also demonstrated that LAB strains were able to produce antioxidant activity in dairy products. The formation of 4–20 kDa peptides was accompanied by elevated radical scavenging activity [229]. *L. plantarum* KFRI 00144, *L. delbrueckii* subsp. *lactis* KFRI 01181, *Bifidobacterium breve* KFRI K-101 and *Bifidobacterium thermophilum* KFRI 00748 were able to efficiently biotransform isoflavone glucosides to their bioactive aglycones during soybean fermentation. Isoflavones are known for their potential bioactive antioxidant properties and radical scavenging capacity. It has been shown that isoflavone glucosides were poorly absorbed in the small intestine compared with their aglycones, so
that soybean fermented by LAB could be regarded as a potent antioxidant and radical scavenging dietary source [230].

LAB are able to alter flavor and taste characteristics of fermented food. Prolonged wheat and rye fermentations performed by LAB resulted in sourdoughs with acidic (*Lactobacillus fermentum* IMDO 130101, *L. plantarum* IMDO 130201 and *Lactobacillus crustorum* LMG 23699), butter-like (*L. amylovorus* DCE 471), or fruity flavor (*L. sakei* CG1). Carbonyls, including alcohols, acids, aldehydes, hydrocarbon-substituted furans, ketones, esters, pyrazines and pyrrolines, are recognized as important bread flavoring agents [231]. Concerning cheese, proteolysis and the subsequent amino acid catabolism are of primary significance for the development of flavor, irrespective of the cheese variety. Amino acids are major precursors for volatile aroma compounds [94]. Taste and flavor of wines are determined by alcoholic and the following malolactic fermentation. Most red and white wines upon malolactic fermentation display more exquisite taste, with an improved bouquet. On the contrary, light red wines and some white wines are characterized by the grape aromas and the vividness which fades with malolactic fermentation [26].

Production of polyols such as mannitol by bacterial fermentation is a promising method. Fermentation process could have several advantages over the chemical synthesis, such as complete conversion of fructose to mannitol, absence of hardly disposable side products, moderate production conditions and no strict need of highly purified substrates [232]. However, mannitol is still produced industrially by high pressure hydrogenation of fructose/glucose mixtures in aqueous solution at high temperature [233]. It is the same case with other polyols [234].

9. Conclusion

LAB represent a versatile group of microorganisms. Owing to their valuable properties, LAB have been used in food production since ancient times. Development of natural sciences led to discovery of LAB as normal part of human and animal microflora. LAB are recognized as safe microorganisms and they are mainly applied in food industry for production of dairy, meat, bread, fish and vegetable products and in medicine as probiotics. LAB are known to synthesize a wide range of compounds consumed in various areas. LAB produce bacteriocins, vitamins, low calorie sugars, EPS and other valuable substances regarded as additives improving safety, quality and flavor of foodstuffs. However, one of the main LAB products is lactic acid used in food processing, pharmaceutics, cosmetics and other industrial sectors. Steadily growing market demand for this commodity urges researchers and manufacturers to seek less expensive substrates for its synthesis. Many studies deal with industrial and household wastes as appropriate sources for lactic acid production.

Ongoing research revealed encouraging LAB application prospects in other fields, such as agriculture, bioremediation of environment, chemical industry and so on. Need in green energy instead of fossil fuels focused keen interest on bacteria as sources of energy, including
LAB. Despite contradictory results, further investigations could resolve problems caused by inhibitory effects of LAB and thus increase biofuel yields.

Author details

Galina Novik1*, Olga Meerovskaya2 and Victoria Savich1

*Address all correspondence to: galina_novik@mbio.bas-net.by

1 National Academy of Sciences of Belarus, Institute of Microbiology, Minsk, Belarus
2 Belarusian Institute of System Analysis and Information Support for Scientific and Technical Sphere, Minsk, Belarus

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