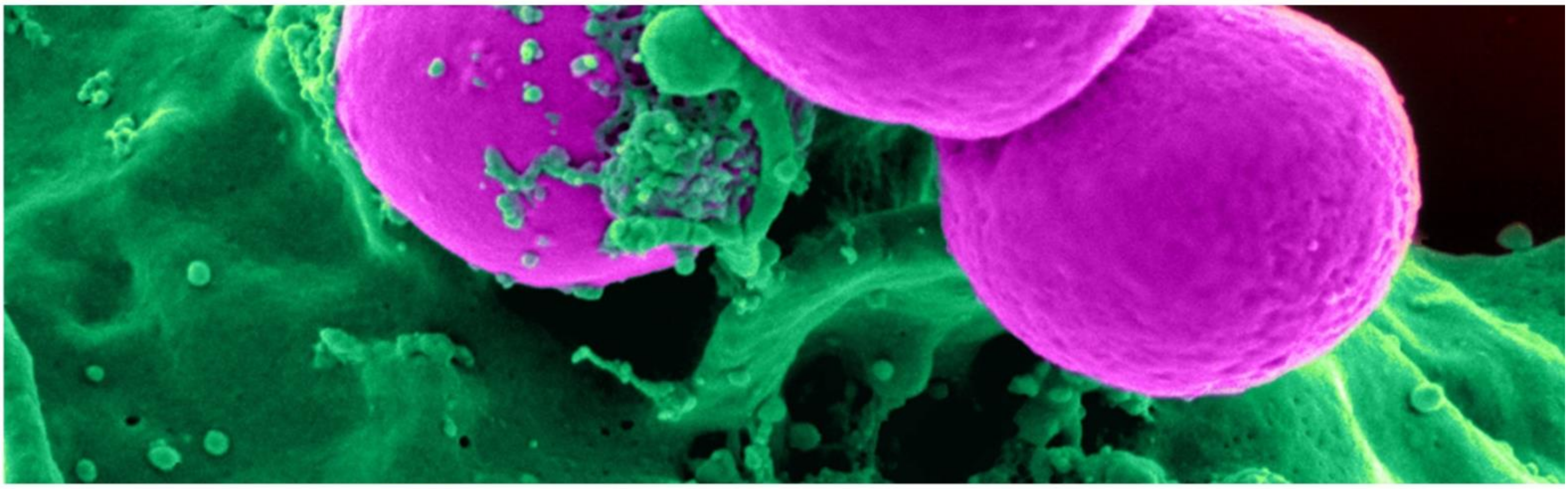
The Institute of Biology
Sri Lanka

Thematic Publication
2023



BIOLOGICAL WEALTH FOR ECONOMIC PROSPERITY

CHAPTER 09

Exploitation of lactic acid bacteria for growth of the food industry

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Abstract

The microbial diversity on earth presents a massive, largely under-exploited genetic and biological resource pool that could be exploited for the recovery of new genes, metabolic pathways, and valuable products. Further, numerous metabolic traits allow microorganisms to perform a variety of essential ecosystem functions, on which several agriculture, food, pharmaceutical, and chemical productions depend. Lactic acid bacteria (LAB) are a heterogeneous group of Gram-positive, non-sporulating, anaerobic, or facultative aerobic cocci or rods, which produce lactic acid as the main end-product of the fermentation of carbohydrates. The LAB used in the food fermentation process had a long history of safe use and are, therefore, referred to as "food grade" or GRAS (Generally Recognized as Safe) microorganisms. The LAB are well known for centuries as safe starter cultures with many numbers of applications including the production of fermented foods and beverages. Moreover, they produce aroma compounds, vitamins, ethanol, bacteriocins, exopolysaccharides, and enzymes of high value. As a result, LAB improve shelf-life, microbial safety, stability, and texture, and contribute to the pleasant sensory profile of the end product in the food industry. Scientific reports support the probiotic effect of LAB extensively including treatment of various gastrointestinal diseases, immunomodulation, anti-diabetic activities, etc. In this chapter, the exploitation of LAB for the development of the food industry is widely discussed in areas such as sustainable agriculture for food and feed production, improving quality and safety of food, biocontrol agents and bio preservatives, for health and wellbeing, biotechnological production of vitamins, organic acids, neutraceuticals, low-calorie sugars, bioactive compounds, etc., biorefining of 'green' crops and food waste management.

Keywords: *Bacteriocins, Biorefining, Bioremediation, Exopolysaccharides, Lactic acid bacteria*

Introduction

Lactic acid bacteria (LAB) are a heterogeneous group of Gram-positive, catalase-negative, anaerobic, or facultatively aerobic, non-sporulating, cocci or rods, which produce lactic acid as the main end-product of the fermentation of carbohydrates. The homofermentative species produce lactic acid (<85%) as the sole end product while the heterofermentative species produce lactic acid, CO_2, and ethanol/acetate. According to the current taxonomic positioning given in the National Centre for Biotechnology Information (NCBI) Taxonomic Browser, LAB has been categorized in the phylum Firmicutes, class Bacilli, and order Lactobacillales. Although LAB include more than 60 genera, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Streptococcus*, *Enterococcus*, and *Weissella* are the main genera involved in food fermentations (Wang et al., 2021). In general, LAB occurs in nutrient-rich habitats and were first isolated from milk. Occurrence of LAB is common and abundant in fermented food products such as meat, milk products, vegetables, beverages, and bakery items. Moreover, they are common inhabitants of soil, water, manure, sewage, silage, and plants, further, are a component of the microbiota on human and animal mucous membranes such as intestines, mouth, skin, and urinary and genital organs (Quinto et al., 2014). Members of this group are of major importance to human health, well-being, and economic activities such as food, chemical, and pharmaceutical industries. Their application in the food fermentation process has a long history of safe use and, therefore, are referred to as "food grade". Consequently, LAB are awarded 'Qualified Presumption of Safety (QPS)' by the European Food Safety Authority and 'Generally Regarded as Safe (GRAS)' status by the US Food and Drug Administration (EFSA, 2010; Qiao et al., 2022). Recently, LAB received great consideration due to their probiotic potential for humans and animals, and important metabolic characteristics including, but not limited to, their ability to degrade indigestible carbohydrates, proteins, and fats, catabolism of amino acids, conversion of non-nutritive and/or unsafe substances present in food and also for producing diverse enzymes, vitamins, antibodies, exopolysaccharides, and various feedstock (Lee et al., 2021; Wang et al., 2021) some of which are detailed below.

Application of LAB in sustainable agriculture for food security

Sustainable agriculture aims at protecting the soil health and the consumer while securing environmental quality. As plant-microbial interactions are integral parts of agriculture, microbial-based agricultural practices are well-defined as briefly discussed below for the roles that they play in maintaining agricultural sustainability leading to food security, which has been defined as all people, at all times, have physical, social, and economic access to sufficient, safe, and nutritious food that meets their food preferences and dietary needs for an active and healthy life (United Nations' Committee on World Food Security).

LAB as biocontrol agents

Nearly 60 billion USD worth of agricultural production (food crops) is lost due to fungal contaminations annually. According to the statistics of the Food and Agriculture Organization (FAO), global mycotoxin (naturally occurring toxins produced by certain fungi) contamination of food produces is around 25%. The ability of LAB to control fungal growth is well documented. Studies have shown that LABs produce substances like reuterin, bacteriocins, diacetyl, reutericyclin, organic acids, acetoin, hydrogen peroxide, etc., with antifungal and antibacterial activity against plant pathogenic microbes, (e.g. members of *Pseudomonas*, *Aspergillus*, *Debaryomyces* *Penicillium* genera), protect some plants from abiotic stress (the negative impact of non-living factors on living organisms in a specific environment), promote growth, perform as plant stimulants and also reduce the *E. coli* counts and their distribution in fermented food and vegetables (Raman et al., 2022).

Some strains of LAB like *Lactococcus lactis* and *Limosilactobacillus reuteri* are vigorous bacteriocin (small peptide units produced by ribosomes with the ability to inhibit the reproduction and growth of numerous bacteria) producers. They are detrimental to microorganisms and, hence, are promising candidates for developing antimicrobials. Further, biosurfactants (surface-active biomolecules) produced by LAB with antifungal, antibacterial and antiviral properties are used in sustainable agriculture (Rooney et al., 2020; Raman et al., 2022). Spoilage of food by molds mainly of *Aspergillus*, *Penicillium*, *Fusarium*, and *Alternaria* genera causes vast economic losses and their toxins such as aflatoxins, ochratoxin, patulin etc., endanger people's health and safety. Filamentous fungi, mostly aspergilli and fusaria, are the key causes of food, crop and animal feed spoilage. Acidic substances produced by LAB fermentation such as lactic acid, acetic acid, propionic acid, phenyllactic acid, and fatty acids reduce the pH level and cause intracellular acidic stress, creating an unfavorable environment for the growth of fungi in food. Control of fungal diseases of cereal crops is important to ensure food and feed safety. Few examples of employing LAB as biocontrol agents in various crops as reported by Li et al. (2020), Raman et al. (2022), and Zhao et al. (2022) are below listed.

01. Controlling Fusarium head blight and pathogenic *Zymoseptoria tritici* and ascomycete fungi causing septoria leaf blotch in wheat
02. Reducing toxic agents produced by filamentous fungi in wheat and maize grains
03. Controlling fruit rot of jackfruit by *Rhizopus stolonifer*
04. Controlling blue mold infection in fresh foods
05. Bio-controlling activity agent toxigenic fungi in table grapes
06. Reducing mycotoxin content in viticulture
07. Antagonism of *Lb. plantarum* species against necrotrophic fungi (*Botrytis cinerea*)
08. Antifungal activity of *Lb. plantarum* IMAU10014 against citrus green rot
09. Antifungal activity of *Lb. pentosus* against filamentous fungi and yeast pathogens
10. Edible films containing 2% LAB prevented banana blackening and prolonged shelf-life
11. Metabolites of LAB neutralized mycotoxin levels and inhibited pathogenic fungal populations in fruit and vegetable crops

Moreover, bio-pesticides like subspecies and strains of *Bacillus thuringiensis*, which are safe, eco-friendly and target specific are receiving huge interest as an alternative to conventional chemical pesticides. For example, metabolites produced by *Lb. sakei* and *Lb. curvatus* tend to kill nematodes (very small, slender worms) and LAB fermented dairy products along with commercial insecticides have elevated the insecticidal activity of the agents (Al-Mahin et al., 2012).

Biostimulants and biofertilizers of LAB origin

Plant growth stimulation and resistance development to abiotic stress by *Lc. lactis* and *Lb. plantarum* is well supported by several authors including Strafella et al. (2021) and Raman et al. (2022). The LAB species are also known to increase crop yield by solubilizing phosphates, accelerating mineral uptake, enhancing organic matter catabolism, decomposing, and bio-stabilizing waste of animal and plant origin. These processes improve the agronomic value of soil by assimilating organic matter, increasing soil structure, aeration, and fertility, neutralizing alkalinity, and endorsing soil moisture retention (Strafella et al., 2021; Raman et al., 2022).

Applications of LAB in food processing, improving food quality and safety

The long history of safe use, GRAS status and, favourable metabolic activities while growing in foods make LAB the most frequently used genera for the production and preservation of foods. As a result, a range of starter, functional and bio-protective, cultures are commercially produced as below discussed.

Production of fermented foods

Fermentation is a first-born biotechnological technique employed in the food production process. Currently, it has become one of the main primary processes of the food industry. Earlier, fermentation was conducted employing undefined cultures with low efficiency and inconsistent quality of the final product. At present, selected, defined starter cultures are used in the industrial fermentations. The frequently used LAB genera in food fermentations include *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Streptococcus*, *Pediococcus*, *Enterococcus,* and *Weissella*. The main application of LAB as starter cultures is for producing many varieties of fermented foods including dairy (e.g. cheese, yoghurt, fermented milk), meat, fish, fruit, vegetable and cereal products. The most frequently used starter cultures containing LAB are shown in Table 1.

Fermentation ensures a longer shelf-life by reducing microbial spoilage and the development of food toxins such as cyanogenic glycoside, glucosinolates, etc., enhancing safety by restraining the transfer of pathogenic microorganisms like *E. coli*, *Staphylococcus aureus* etc. while improving organoleptic properties (the features of food, that create specific experiences via the senses, e.g. taste, sight, smell, and touch) and digestibility. Fermentation also increases the activity of microbial enzymes such as amylases, lipases, proteases, and phytases. These enzymes modify the raw material through the hydrolysis of polysaccharides, proteins, and fat hence, while improving the nutritional value of the raw material by degrading anti-nutritive compounds, like phytic acid and tannins (Zapasnik, 2022).

Table 1. LAB used as starter cultures in fermented food products.

Product category	LAB species
Dairy products	
Cheese (Mesophilic starter)	*Lc. lactis* ssp. *Lactis, Lc. lactis* ssp. *Cremoris, Lc. lactis* ssp. *lactis* var. *diacetylactis, Leuc. mesenteroides* ssp. *Cremoris*
Cheese (Thermophilic starter)	*S. thermoplillus, Lb. delbrueckii* ssp. *Bulgaricus, Lb. helveticus, Lb. delbrueckii* ssp. *lactis*
Cheese (Mixed starter)	*Lc. lactis* ssp. *Lactis, Lc. lactis* ssp. *Cremoris, S. thermoplillus*
Yoghurt	*Lb. delbrueckii* ssp. *bulgaricus, S. thermophilus*
Fermented milks	*Lb. delbrueckii* ssp. *bulgaricus, S. thermophilus Lb. casei, Lb. acidophilus, Lb. rhamnosus, Lb. johnsonii*
Yakult	*Lb. casei* ssp. *Casei*
Acidophilus milk	*Lb. acidophilus*
Kefir	*Leuc. Mesenteroides Lc. lactis* ssp. *lactis, Lc. lactis* ssp. *lactis var. diacetylactis, Leuc. menesteroides*
Fermented meat products	
Dry sausages	*Lb. sakei, Lb. curvatus, Lb. plantarum, Lb. pentosus, Lb. casei,P. pentosaceous, P. acidilactici*
Greek dry fermented sausages	*Lb. sakei, Lb. plantarum, Lb. curvatus, Lb. pentosus, Lc. lactis* ssp. *lactis, W. hellenica*
Fermented fish products	
Thai fish	*Lb. plantarum, Lb. reuteri*
Pickled fruits and vegetables	
Cabbage (Sauerkraut)	*Leuc. mesenteroides, Lb. plantarum, Lb. brevis, Lb. fermentum*
Cucumber	*Lb. brevis, Lb. plantarum, Lb. pentosus, Lb. acidophilus, Lb. fermentum, Leuc. Mesenteroides*
Olives	*Lb. brevis, Lb. plantarum, Lb. pentosus*
Fermented cereal products	
Sourdough	*Lb. brevis, Lb. hilgardii, Lb. sanfransiscensis, Lb. farciminis, Lb. fermentum, Lb. brevis, Lb. plantarum, Lb. amylovorus, Lb. reuteri, Lb. pontis, Lb. panis*
Kimchi	*Leuc. mesenteroides, Lb. plantarum, Lb. kimchii*

Note: *Lc. Lactococcus, Lb. Lactobacillus, Leuc. Leuconostoc, P. Pediococcus, S. Streptococcus, W. Weissella*

Source: Bintsis, 2018a

Food preservation and safety

Preservatives of biological origin (derived from plants, animals, and bacteria) are now becoming popular in the global food additive market, due to their natural origin, non-toxicity or low toxicity, high efficiency, and also their ability to preserve the original flavour of the food (Zhao et al., 2022). The LAB produce a range of biologically active metabolites with numerous antimicrobial activities (lactic acid, acetic acid, hydrogen peroxide, low molecular weight substances such as fatty acids, diacetyl, reutericyclin, reuterin, antifungal compounds like propionate, phenyl lactate, hydroxyphenyl lactate and bacteriocins), hence, are well known for their effective bio-preservative properties. The LAB also neutralize mycotoxin produced by filamentous fungi by producing anti-mycotoxinogenic metabolites with GRAS status received from US Food and Drug Administration (Zapasnik, 2022).

Functional food industry

The LABs own proven therapeutic properties that are essential for protecting and improving human health. The LAB increase the bioavailability of nutrients, produce compounds having antioxidant activity, and also synthesize vitamins (biotin, cobalamin, folates). Antioxidant activity is related to their ability to transform phenolic acids to biologically active forms, positive effect on the content of ascorbic acid, and conversion of individual diet components into group B and K vitamins.

A correlation between high antioxidant activity and anticarcinogenic properties of bacterial lysate is also reported (Zapasnik, 2022). Several LAB genera have also been recognized as good probiotics (the FAO/WHO defines probiotics as "live microorganisms which when administered in adequate amounts confer a health benefit on the host) and the global probiotics market is characterized by three main applications as listed below.

1. Probiotics in foods and beverages (e.g. dairy, non-dairy, cereal, baked food, fermented vegetable, fruit, and meat products)
2. Probiotic dietary supplements (e.g. single cell protein, nutraceuticals, specialty nutrients, and infant formula)
3. Animal feed probiotics (e.g. substitutes for antibiotics and growth stimulants).

According to Zoumpopoulou et al. (2018), the US and EU market value in these sectors was estimated to be over USD 1.8 billion and 630 million, respectively, in 2017. An increasing number of food and feed companies worldwide are, therefore, trying to utilize LAB in their products to meet customer demand, improve product quality and diversify their product lines. The extensively discussed probiotics of LAB origin include *Lb acidophilus*, *Lb. casei*, *Lb. fermentum*, *Lb. gasseri*, *Lb. johnsonii*, *Lb. paracasei*, *Lb. plantarum*, *Lb. rhamnosus,* and *Lb. salivarius*. The other probiotic LAB species include *S. thermophilus*, *Bifidobacterium lactis*, *B. longum* and *B. breve*. The beneficial effects of these probiotics are mostly strain-specific and include a contribution to the healthy gut microbiota, treatment of various gastrointestinal diseases, maintaining a healthy immune system, controlling pathogenic bacteria, supporting the health of the reproductive tract, oral cavity, lungs, skin, circulatory system and gut–brain axis (Hill, 2014; Bintsis, 2018a). Therefore, a large number of LAB are employed as commercial probiotic products with proven health-beneficial properties (Table 2).

Table 2. Commercial probiotic products available in the market.

Brand/trade name	Food type	Sources/ strains	Manufacturer company	Country
Aciforce	Freeze-dried product	*Lc. lactis, Lb. acidophilus, En. faecium, B.bifidum*	Biohorma	Netherlands
Actimel	Yoghurt drink	*Lb. casei immunitas*	Danone	France
Bacilac	Freeze-dried product	*Lb.acidophilus, Lb.rhamnosus*	THT	Belgium
Bififlor	Freeze-dried product	*Lb.acidophilus, Lb. rhamnosus, B.bifidum*	Eko-Bio	Netherlands
Hellus	Dairy product	*Lb. fermentum* ME-3	Tallinna Piimatööstuse AS	Estonia
Proflora	Freeze-dried product	*Lb acidophilus, Lbdelbrueckii* subsp. *bulgaricus, S.thermophilus, Bifidobacterium* spp.	Chefaro	Belgium
Provie	Fruit drink	*Lb plantarum*	Skanemejerier	Sweden
ProViva	Fruit drink and yoghurt	*Lb.plantarum*	Skanemejerier	Sweden
Yakult	Milk drink	*Lb. casei Shirota*	Yakult	Japan

Lc. Lactococcus, Lb. Lactobacillus, Leuc. Leuconostoc, P. Pediococcus, S. Streptococcus, En. Enterococcus, B. Bifidobacterium

Source: Mishra et al. (2015)

Industrially valuable products synthesized by LAB

The LABs produce a variety of valuable products including, but not limited to, organic acids, amino acids, short-chain fatty acids (SCFA), amines, bacteriocins, vitamins (folic acid, riboflavin, vitamin C, pyridoxal), nutraceuticals, low-calorie sugars (mannitol), bioactive compounds (exopolysaccharides, antioxidants), flavour substances (2,3-butanedione, 2,3-pentane-dione). As a result, in recent years, LAB have attracted more attention from science and industry and shown a diversity of extended applications in the food-related industries as discussed below.

Organic acids

Organic acids produced by LAB include lactic acid (main product), acetic, formic, succinic and citric which are of industrial value. Organic acids are extensively applied in numerous industries due to their versatile use as monomers and starting materials for biodegradable polymers, food supplements, and bio-based materials for bio-refinery (Wang, 2021; Ibrahim et al., 2021). Recently, lactic acid has gained more interest due to its utilization in the manufacture of poly lactic acid, which is a green polymer, a substitute for petroleum-derived plastics. It is also applied in medicine for the regeneration of damaged tissues and in sutures, repairs and implants. Moreover, lactic acid is used as flavourings, acidulants, buffering agents, and inhibitors of bacterial spoilage in a wide variety of processed foods and also in chemical, textile, and pharmaceutical industries. Different esters of lactic acid are frequently used as emulsifying agents in baking foods (Lee et al., 2021).

Bacteriocins

Antibiotic resistance is escalating and threatening humans and animals worldwide. Bacteriocins synthesized by LAB could be used as an alternative to antibiotics (Wang, 2021; Ibrahim et al., 2021). Bacteriocins of LAB origin are considered ideal for food industry applications based on characteristics including GRAS status, odourless and colourless nature, preserving the organoleptic qualities of food, easy clearance by the proteolytic enzymes, etc. One of the best-known bacteriocin is nisin, produced by LAB species including *Lactococcus lactis* ssp. *lactis* which are used as permitted food additives in nearly 50 countries around the globe, mainly in canned foods, dairy industry and also for controlling mastitis (Lee et al., 2021).

Exopolysaccharides

Extracellular polymers produced by microorganisms when composed of sugars are referred to as exopolysaccharides (EPS). The LAB have the ability to secrete biopolymers such as xanthan, gellan, dextran, pullulan, scleroglucan, etc. Due to their composition, structure, and physical properties, EPS are used in different food and pharmaceutical industries. For example, EPS improves the viscosity and texture during milk fermentation and these biopolymers act as thickeners, stabilizers, gelling, and viscosifying agents, emulsifiers, and fat replacers in low-caloric foods. The EPS are also recognized as prebiotics (natural substances in some foods that boost the growth of beneficial bacteria in the gut). Further, EPS prompt positive physiological reactions such as lowering cholesterol levels, reducing the formation of pathogenic biofilms and adhesion to epithelial cells and anti-carcinogenic, immune-modulating and prebiotic effects (Nath and Malik, 2016).

Application of LAB in food waste management

An amount of 1,300 billion tons of food waste (FW) per year is produced globally which is one-third of the total global food production (FAO, 2013). Thus, the global community is driving towards valorising FW while taking steps for its reduction. The FW possesses a huge potential for fermentation due to its vast yield and organic content. Among a number of FW fermentation methods such as fermentation towards Mixed Volatile Fatty Acids (VFA), butyric acid, acetic acid, and ethanol production, fermentation them into lactic acid using LAB is the method that requires the least interventions. Compared to chemical synthesis, lactic acid fermentation is a better technique to produce the acid due to the biological process' low energy requirements, low cost of substrates, and low reaction temperature (Wang et al., 2020; Raman et al., 2022). There was an excessive need for lactic acid of 1.2 million tons in 2016 with an anticipated annual growth rate of 16.2% till 2025 (Esteban et al., 2018).

In addition, it has been found that the use of undefined mix cultures of LAB can be more beneficial than using pure cultures due to reasons such as no sterilization requirements, improved adaptive capacity host environment, and the capability of utilizing mixed substrates (Nunes et al., 2017).

Application of LAB in the animal feed and livestock health sectors

Based on the functions of the additives used in the production of animal feed, they can be categorized into several groups such as nutritional, zootechnical, and sensorial (additives affecting the organoleptic properties of animal products). Among them, zootechnical animal feed addresses animal health through feedstuff. The use of LAB as a sensorial or nutritional feed is found to be occasional. Therefore, parallel to the use of LAB as probiotics for human consumption, their usage in animal products has drawn attention. It is true that most of the time LAB are employed as probiotics (*Lb. acidophilus, Lb. bulgaricus Lb. reuteri,* etc.) to enhance animal health and guard against illnesses. The majority of them fall under the category of "zootechnical feed" and focus on benefits for the gastrointestinal tract. The few most significant benefits of using probiotic LAB are enhancing growth rate, disease control, body weight management, and milk and egg production as summarized in Table 3.

Table 3: Benefits of LAB towards animal health.

Category	LAB species	Benefit	Reference
Ruminant	*Lactobacillus gasseri*	*In vitro* inhibitory activity against *Staphylococcus aureus*	Otero et al., 2006
Ruminant	*Pediococcus* spp., *Leuconostoc* spp	Act against urogenital infections	Wang et al., 2013a
Swine	*Lactobacillus sobrius*	Weight gain improvement & reduction in the amount of enterotoxigenic *E. coli* in the ileum, intestinal defenses & modulation of gut microbiota.	Jakava-Viljanen et al., 2008
Poultry	*Lactobacillus* spp.	Increase recovery of broiler chicks after infected with *Salmonella enteritidis*	Higgins et al., 2008

Future prospects of LAB

Due to the safety for human and animal consumption and metabolic versatility (including industrial-scale fermentations), LAB are gaining attention towards novel uses such as (industrial production of green chemicals, fuels, enzymes etc.). Analysing complete genomic information of LAB is at the top among the future prospects to enhance and improve the existing applications (Peng et al., 2022). Thus, one of the major focuses is using whole-genome sequencing (WGS) of individual LAB strains to evaluate the product's safety by identifying potential virulence genes and other factors that could have a detrimental influence on health through food. Two major examples of using genetic modifications in the food industry are the expanding substrate utilization performance of *Lc. lactis* by expressing α-amylase-encoding gene AmyA from *Streptococcus bovis* and improving the metabolite production of *Lb. plantarum* by co-expressing pyruvate carboxylase and phosphoenolpyruvate carboxykinase (Okano et al., 2007; Tsuji et al., 2013).

The use of bioinformatics in WGS is one of the most technically suitable & cost-effective recommended approaches. It can be used to increase understanding on LAB strains in the food safety (Mendoza et al., 2023). However, producing genetically modified LAB using WGS is severely constrained in terms of food applications due to laws and regulations as well as the unfavourable consumer perception of GMOs and their metabolites (Bintsis, 2018b). At the same time, being an alternative for chemical preservatives and many other food-related uses, the applications of LAB will be further enhanced and diversified in the coming decades with the use of WGS.

Necessity of employing LABs for the growth of Sri Lankan food industry

In this chapter, we briefly discussed numerous applications of beneficial LAB for the growth of agriculture and food industry, which plays a major role in sustainable economic development. However, Sri Lanka is still far from fully exploiting and utilizing these valuable microbial resources as extremely limited work has been done on the indigenous microbial strains with industrial prospects in Sri Lanka. As a result, Sri Lanka does not have a starter culture production facility or a functioning industrial culture collection for the country, apart from a depository of an industrially beneficial LAB and yeast isolates obtained from Sri Lankan dairies within the Industrial Technology Institute (ITI) under the Ministry of Industries. Consequently, most basic food industries (bakery foods, production of alcoholic beverages, production of fermented dairy/meat/vegetable foods such as yoghurt, curd, cheese, pickles and salami etc.) completely depend on imported, genetically modified, freeze-dried cultures and/or undefined starters with unpredictable performances. Our industries (dairy industry is on the top of this list) using imported cultures or undefined starters face huge economic losses, due to changes in import/export policies which restricts the importation of certain cultures after designing and establishing production processes, loss of viability and functionality during shipments, price, inability to utilize for multiple applications, not fitting for household and small scale food processing applications, unpredictable bottlenecks in global supplies like covid pandemic, etc.

Furthermore, none of our industries are utilizing microorganisms for the production of lactic acid, antibiotics, enzymes, vitamins, single cell proteins, amino acids, food grade/pharmaceutical solvents, chemicals, etc. which have huge economic prospects. Further, a very limited range of fermented foods are available for the Sri Lankan consumers and the functional prospects of those products are not scientifically proven. Hence, numerous opportunities exist for market oriented new product developments to enhance the colonic and general health status of the consumers and to satisfy the market demand through the introduction of locally developed indigenous functional starter cultures and their technological application process design (Rajawardana et al., 2020). Moreover, bovine mastitis accounting for considerable economic losses in dairy industry is widespread in dairy herds all over the world, including Sri Lanka. The overall prevalence of sub clinical mastitis in major milk-producing areas of Sri Lanka was 27.3%, and *Staphylococcus aureus* was the most common pathogen identified. Unhygienic milking practices, poor health management, and other environmental and farming conditions mainly lead to the high prevalence of mastitis (Ranasinghe et al., 2021). As discussed earlier, nisin produced by LAB is highly effective for controlling mastitis-causing pathogens like *Staphylococcus aureus*. In addition, being a country that produces tons of agricultural waste, and a country that anything can grow, bioremediation is mostly limited only to research so far. Therefore, there is an urgent need to fully and immediately exploit existing microbial biotechnologies to strengthen food value chain to maximize food security, safety and of waste management. This may result in better process control, enhanced food security, safety and quality, and reduction of economic losses.

Conclusion and perspectives

The LAB are the most commonly used microorganisms in the food industry. Their safe and favorable metabolic activities and GRAS approved beneficial metabolites are gaining huge attention hence, a range of starter, functional, bio-protective and probiotic cultures with desirable properties are commercially produced worldwide. The current advances in the biotechnology, nanotechnology and genetics, have provided new insights and applications for the LAB in numerous sectors including, but not limited to, agriculture, food, pharmaceutical and chemical industries. Although analyzing complete genomic information of LAB is at the top among the future prospects to enhance and improve the existing application. However, producing genetically modified LAB is severely constrained in terms of food applications due to laws and regulations of responsible authorities including FDA, European Union (EU) and World Trade Organization (WTO). The LAB being an alternative for chemical preservatives and many other food-related uses, the applications of LAB will be further enhanced and diversified in the coming decades with the use of WGS.

References

Al-Mahin, A & Sonomoto, K 2012. Nisin tolerance of DnaK-overexpressing *Lactococcus lactis* strains at 40 °C. *American Journal of Biochemistry and Molecular Biology,* 2(3), pp.157–166.

Bintsis, T 2018a. Lactic acid bacteria: their applications in foods. *Journal of Bacteriology & Mycology*, 6(2), pp. 89–94.

Bintsis, T 2018b. Lactic acid bacteria as starter cultures: An update in their metabolism and genetics. *AIMS Microbiology*, 4, 665.

Esteban, J & Ladero, M 2018. Food waste as a source of value-added chemicals and materials: a biorefinery perspective. *International Journal of Food Science and Technology*, 53, pp. 1095–1108.

FAO, 2013. Food wastage footprint. Impact on Natural Resources; Summary Report; FAO: Rome, Italy.

Higgins, SE, Higgins, JP, Wolfenden, AD, Henderson, SN, Torres-Rodriguez, A, Tellez, G, & Hargis, B 2008. Evaluation of a *Lactobacillus*-based probiotic culture for the reduction of *Salmonella enteritidis* in neonatal broiler chicks. *Poultry Science*, 87, pp. 27–31.

Hill, C, Guarner, F, Reid, G. et al., 2014. The International Scientific Association for Probiotics and Prebiotics Consensus Statement on the Scope and Appropriate Use of the Term Probiotic. *Nature Reviews. Gastroenterology & Hepatology*, 11(8), pp. 506–514.

Ibrahim, SA, Ayivi, RD, Zimmerman, T, Siddiqui, SA, Altemimi, AB, Fidan, H, Esatbeyoglu, T & Bakhshayesh, RV 2021. Lactic acid bacteria as antimicrobial agents: Food safety and microbial food spoilage prevention. *Foods*, 10, 3131.

Jakava-Viljanen, M, Murros, A, Palva, A & Bjorkroth, KJ 2008. *Lactobacillus sobrius* is a later synonym of *Lactobacillus amylovorus*. *International Journal of Systematic and Evolutionary Microbiology*, 58, pp. 910–913.

Lee, J, Jeon, S, Yoo, Y & Kim, H 2021. Some important metabolites produced by Lactic Acid bacteria originated from Kimchi. *Foods*, 10, 9.

Li, Z, Wang, L, Xie, B, Hu, S, Zheng, Y & Jin, P 2020. Effects of exogenous calcium and calcium chelant on cold tolerance of postharvest loquat fruit. *Scientia Horticulturae*, 269(25):109391.

Mendoza, RM, Kim, SH, Vasquez, R, Hwang, IC, Park, YS, Paik, HD, Moon, GS & Kang, DK 2023. Bioinformatics and its role in the study of the evolution and probiotic potential of lactic acid bacteria. *Food Science and Biotechnology*, 32,4, pp 389-412.

Mishra, V, Shah, C, Mokashe, N, Chavan, R, Yadav, H & Prajapati, J 2015. Probiotics as potential antioxidants: A systematic review. *Journal of Agricultural and Food Chemistry*, 63(14), pp. 3615-3626.

Nath, U. & Malik, RK 2016. Screening and characterization of exopolysaccharide producing *Lactobacillus* sp. *Indian Journal of Dairy Science*, 69(2), pp. 154-164.

Nunes, LV, de Barros Correa, FF, de Oliva Neto P, Mayer, CRM, Escaramboni, B, Campioni, TS et al. 2017. Lactic acid production from submerged fermentation of broken rice using undefined mixed culture. *World Journal of Microbiology and Biotechnology*, 33, 79.

Okano, K, Kimura, S, Narita, J et al. 2007. Improvement in lactic acid production from starch using alpha-amylase secreting Lactococcus lactis cells adapted to maltose or starch. *Applied Microbiology and Biotechnology*, 75, pp. 1007–13.

Otero, MC, Morelli, L & Nader-Macias, ME 2006. Probiotic properties of vaginal lactic acid bacteria to prevent metritis in cattle. *Letters in Applied Microbiology*, 43, pp. 91–97.

Peng, X, Ed-Dra, A & Yue, M 2022. Whole genome sequencing for the risk assessment of probiotic lactic acid bacteria. *Critical Reviews in Food Science and Nutrition*, pp.1-19.

Qiao, N, Wittouck, S, Mattarelli, P, Zheng, J, Lebeer, S, Felis, GE & Gänzle, MG 2022. After the storm-Perspectives on the taxonomy of Lactobacillaceae. *JDS Communications*, 3,3, pp. 222-227.

Quinto, EJ, Jiménez, P, Caro, I, Tejero, J, Mateo, J & Girbés, T 2014. Probiotic Lactic Acid Bacteria: A Review. *Food and Nutrition Sciences*, 05, pp. 1765-1775.

Rajawardana, DU 2020. Sri Lankan dairy microbiota; biodiversity of bacterial populations and multifunctional applications of probiotic lactic acid bacteria and yeast. Ph.D. thesis, University of Colombo, Sri Lanka.

Raman, J, Kim, JS, Choi, KR, Eun, H, Yang, D, Ko, YJ & Kim, SJ 2022. Application of Lactic Acid Bacteria (LAB) in sustainable agriculture: Advantages and limitations. *International Journal of Molecular Sciences*, 23,14,7784.

Ranasinghe, RMSBK., Deshapriya, RMC, Abeygunawardana, DI, Rahularaj, R, & Dematawewa, CMB 2021. Subclinical mastitis in dairy cows in major milk-producing areas of Sri Lanka: Prevalence, associated risk factors, and effects on reproduction. *Journal of Dairy Science,* 104(12), 12900-12911.

Rooney, WM, Grinter, RW, Correia, A, Parkhill, J, Walker, DC & Milner, JJ 2020. Engineering bacteriocin-mediated resistance against the plant pathogen *Pseudomonas syringae*. *Plant Biotechnology Journal*, 18(5), pp. 1296–1306.

Strafella, S, Simpson, DJ, Yaghoubi Khanghahi, M, De Angelis, M, Ganzle, M, Minervini, F & Crecchio, C 2021. Comparative genomics and in vitro plant growth promotion and biocontrol traits of lactic acid bacteria from the wheat rhizosphere. *Microorganisms*, 9(78).

Tsuji, A, Okada, S, Hols, P & Satoh, E 2013. Metabolic engineering of *Lactobacillus plantarum* for succinic acid production through activation of the reductive branch of the tricarboxylic acid cycle. *Enzyme and Microbial Technology*, 53,2, pp. 97-103.

Wang, Q, Li, H, Feng, K & Liu, J 2020. Oriented fermentation of food waste towards high-value products: A review. *Energies*, 13(21), 5638.

Wang, Y, Ametaj, BN, Ambrose, DJ & Ganzle, MG 2013. Characterization of the bacterial microbiota of the vagina of dairy cows and isolation of pediocin-producing *Pediococcus acidilactici*. *BMC Microbiology*, 13, 19.

Wang, Y, Wu, J, Lv, M, Shao, Z, Hungwe, M, Wang, J, Bai, X, Xie, J, Wang, Y & Geng W 2021. Metabolism Characteristics of Lactic Acid Bacteria and the Expanding Applications in Food Industry. *Front Bioeng Biotechnol*, 9, 612285.

Zapasnik, A, Sokołowska, B & Bryła, M 2022. Role of lactic acid bacteria in food preservation and safety. *Foods*, 11, 1283.

Zhao, S, Hao, X, Yang, F, Wang, Y, Fan, X & Wang, Y 2022. Antifungal activity of *Lactobacillus plantarum* ZZUA493 and its application to extend the shelf life of Chinese steamed buns. *Foods*,11, 195.

Zoumpopoulou, G, Kazou, M, Alexandraki, V, Angelopoulou, A, Papadimitriou, K, Pot, B & Tsakalidou, E 2018. Probiotics and prebiotics: an overview on recent trends. In D. Di Gioia & B. (Ed.) Biavati Probiotics and prebiotics in animal health and food safety (pp. 1-34). Springer, Cham.