

Lactic acid bacteria: their applications in foods

Abstract

Lactic acid bacteria (LAB) are heterogenous group of bacteria which plays a significant role in a variety of fermentation processes. They ferment food carbohydrates and produce lactic acid as the main product of fermentation. In addition, degradation of proteins and lipids and production of various alcohols, aldehydes, acids, esters and sulphur compounds contribute to the specific flavour development in different fermented food products.

The main application of LAB is as starter cultures, with an enormous variety of fermented dairy (ie. cheese, yoghurt, fermented milks), meat, fish, fruit, vegetable and cereal products. Besides, they contribute to the flavour, texture and nutritional value of the fermented foods, and thus they are used as adjunct cultures. Acceleration of cheese maturation, enhancement of yoghurt texture with the production of exo polysaccharides and control of secondary fermentations in the production of wine are some examples. The production of bacteriocins and antifungal compounds has lead to the application of bio-protective cultures in certain foods. Moreover, the well-documented health-promoting properties of certain LAB have lead to the addition of selected strains, in combination with bifidobacteria, as probiotic cultures with various applications in food industry.

Keywords: lactic acid bacteria, applications, fermented foods

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Bintsis T

Department of Agricultural Technology, TEI of West Macedonia, Greece

Correspondence: Bintsis T, Department of Agricultural Technology, TEI of West Macedonia, 50100 Kozani, Greece, Tel +30 69 4872 1720, Fax +30 24 6302 4995, Email tbintsis@gmail.com, bintsis@kastoria.teiwm.gr

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Introduction

Lactic acid bacteria (LAB) play an important role in food, agricultural, and clinical applications. The general description of the bacteria included in the group is gram-positive, nonsporing, nonrespiring cocci or rods, which produce lactic acid as the major end product during the fermentation of carbohydrates.¹ The common agreement is that there is a core group consisting of four genera; *Lactobacillus*, *Leuconostoc*, *Pediococcus* and *Streptococcus*. Recent taxonomic revisions have proposed several new genera and the remaining group now comprises the following: *Aerococcus*, *Alloiococcus*, *Carnobacterium*, *Dolosigranulum*, *Enterococcus*, *Globicatella*, *Lactococcus*, *Oenococcus*, *Tetragenococcus*, *Vagococcus*, and *Weissella*.² Their importance is associated mainly with their safe metabolic activity while growing in foods utilising available sugar for the production of organic acids and other metabolites. Their common occurrence in foods along with their long-lived uses contributes to their natural acceptance as GRAS (Generally Recognised as Safe) for human consumption.³ The EFSA's 'Panel on Biological Hazards (BIOHAZ)' has concluded that for the fermenting bacteria associated with food, whether resistant to antibiotics or not - with the possible exception of enterococci - there is no evidence for any clinical problem.⁴ However, they can act as a reservoir for transferable resistance genes. Strains with genes transferable in such a way could enter the food chain and increase the probability of a transfer to food associated intestinal pathogenic organisms.

The three main pathways which are involved in the manufacture and development of flavour in fermented food products are 1) glycolysis (fermentation of sugars), 2) lipolysis (degradation of fat) and 3) proteolysis (degradation of proteins).^{1,5-9} Lactate is the main product generated from the metabolism of carbohydrates and a fraction of the intermediate pyruvate can alternatively be converted

to diacetyl, acetoin, acetaldehyde or acetic acid (some of which can be important for typical yogurt flavours). The contribution of LAB to lipolysis is relatively little, but proteolysis is the key biochemical pathway for the development of flavour in fermented foods.^{10,11} Degradation of such components can be further converted to various alcohols, aldehydes, acids, esters and sulphur compounds for specific flavour development in fermented food products.^{10,11}

The genetics of the LAB have been reviewed¹²⁻¹⁸ and complete genome sequences of a great number of LAB have been published¹⁹ since 2001, when the first genome of LAB (*Lactococcus lactis* ssp. *lactis* IL1403) was sequenced and published.²⁰

Applications of LAB

Starter cultures for fermented foods

Fermented foods are produced through fermentation of certain sugars by LAB and the origins of them are lost in antiquity. The most commonly LAB used as starter cultures in food fermentations are shown in Table 1. It is well-known that the greatest proportion of them belong to the category of dairy products, namely cheese, yoghurt, fermented milks, while fermented meat products, fish products, pickled vegetables and olives and a great variety of cereal products are manufactured, nowadays, using starter cultures. These products, were produced in the past through back slopping and the resulting product characteristics depended on the best-adapted strains dominance, whereas, the earliest productions of them were based on the spontaneous fermentation, resulting from the development of the microflora naturally present in the raw material and its environment. Today, the majority of fermented foods are manufactured with the addition of selected, well defined, starter cultures with well characterized traits, specific for each individual product. For a detailed classification of starter cultures see.²¹⁻²³

Table 1 Lactic acid bacteria used as starter cultures in the production of some fermented food products

Product	Genera of LABI	Reference
Dairy products		
Cheese (Mesophilic starter)	<i>Lc. lactis</i> ssp. <i>lactis</i> <i>Lc. lactis</i> ssp. <i>cremoris</i> <i>Lc. lactis</i> ssp. <i>lactis</i> var. <i>diacetylactis</i> <i>Leuc. mesenteroides</i> ssp. <i>cremoris</i>	22
Cheese (Thermophilic starter)	<i>S. thermophilus</i> <i>Lb. delbrueckii</i> ssp. <i>bulgaricus</i> <i>Lb. helveticus</i> <i>Lb. delbrueckii</i> ssp. <i>lactis</i>	22
Cheese (Mixed starter)	<i>Lc. lactis</i> ssp. <i>lactis</i> <i>Lc. lactis</i> ssp. <i>cremoris</i> <i>S. thermophilus</i>	22
Yogurt	<i>Lb. delbrueckii</i> ssp. <i>bulgaricus</i> , <i>S. thermophilus</i>	22
Fermented milks	<i>Lb. delbrueckii</i> ssp. <i>bulgaricus</i> , <i>S. thermophilus</i> <i>Lb. casei</i> , <i>Lb. acidophilus</i> , <i>Lb. rhamnosus</i> , <i>Lb. johnsonii</i>	22
Yakult	<i>Lb. casei</i> ssp. <i>casei</i>	22
Acidophilus milk	<i>Lb. acidophilus</i>	22
Butter and buttermilk	<i>Lc. lactis</i> ssp. <i>lactis</i> , <i>Lc. lactis</i> ssp. <i>lactis</i> var. <i>diacetylactis</i> , <i>Lc. lactis</i> ssp. <i>cremoris</i> , <i>Leuc. mesenteroides</i> ssp. <i>cremoris</i>	22
Kefir	<i>Lb. kefir</i> , <i>Lb. kefirifaciens</i> , <i>Lb. brevis</i> , <i>Lb. plantarum</i> , <i>Lb. paracasei</i> ssp. <i>paracasei</i> , <i>Lc. lactis</i> ssp. <i>lactis</i> , <i>Leuc. mesenteroides</i>	62
Trahanas	<i>Lc. lactis</i> ssp. <i>lactis</i> , <i>Lc. lactis</i> ssp. <i>lactis</i> var. <i>diacetylactis</i> , <i>Leuc. mesenteroides</i> ssp. <i>cremoris</i> , <i>Lb. delbrueckii</i> ssp. <i>lactis</i> , <i>Lb. casei</i> , <i>Lb. delbrueckii</i> ssp. <i>bulgaricus</i> and <i>Lb. Acidophilus</i>	63
Fermented meat products		
Dry sausages	<i>Lb. sakei</i> , <i>Lb. curvatus</i> , <i>Lb. plantarum</i> , <i>Lb. pentosus</i> , <i>Lb. casei</i> , <i>P. pentosaceus</i> , <i>P. acidilactici</i>	64,65
Salami Milano	<i>Lb. sakei</i> , <i>Lb. plantarum</i>	66
Salame Piacentino	<i>Lb. acidophilus</i> , <i>Lb. helveticus</i> , <i>Lb. sakei</i> , <i>Lb. antri</i> , <i>Lb. oris</i> , <i>Lb. vaginalis</i> , <i>Lb. brevis</i> , <i>Lb. panis</i> , <i>Lb. versmoldensis</i> , <i>Lb. zeae</i> , <i>Lb. curvatus</i> , <i>Lb. paralimentarius</i> , <i>Lb. frumenti</i> , <i>Lb. plantarum</i> , <i>Lb. graminis</i> , <i>Lb. reuteri</i>	67
Greek dry fermented sausages	<i>Lb. sakei</i> , <i>Lb. plantarum</i> , <i>Lb. curvatus</i> , <i>Lb. pentosus</i> , <i>Lc. lactis</i> ssp. <i>lactis</i> , <i>W. hellenica</i> , <i>W. paramesenteroides</i> , <i>W. viridescens</i> , <i>W. minor</i>	67
Chorizo	<i>Lb. brevis</i> , <i>Lb. curvatus</i> , <i>Lb. sakei</i> , <i>Lc. lactis</i> , <i>P. acidilactici</i> , <i>P. pentosaceus</i> , <i>Leuc. mesenteroides</i>	67
Fermented fish products		
Thai fish	<i>Lb. plantarum</i> , <i>Lb. reuteri</i>	68
Pickled fruits and vegetables		
Cabbage (Sauerkraut)	<i>Leuc. mesenteroides</i> , <i>Lb. plantarum</i> , <i>Lb. brevis</i> , <i>Lb. fermentum</i>	69
Cucumber	<i>Lb. brevis</i> , <i>Lb. plantarum</i> , <i>Lb. pentosus</i> , <i>Lb. acidophilus</i> , <i>Lb. fermentum</i> , <i>Leuc. Mesenteroides</i>	70,71
Olives	<i>Lb. brevis</i> , <i>Lb. plantarum</i> , <i>Lb. pentosus</i>	72,73
Fermented cereal products		
Sourdough	<i>Lb. brevis</i> , <i>Lb. hilgardii</i> <i>Lb. sanfransiscensis</i> , <i>Lb. farciminis</i> , <i>Lb. fermentum</i> , <i>Lb. brevis</i> , <i>Lb. plantarum</i> , <i>Lb. amylovorus</i> , <i>Lb. reuteri</i> , <i>Lb. pontis</i> , <i>Lb. panis</i> , <i>Lb. alimentarius</i> , <i>W. cibaria</i>	74,75
Kimchi	<i>Leuc. mesenteroides</i> , <i>Lb. plantarum</i> , <i>W. kimchii</i> sp. nov., <i>Lb. kimchi</i> , <i>Lb. sakei</i> , <i>W. koreensis</i>	76–78
Bushera	<i>Lb. plantarum</i> , <i>Lb. paracasei</i> ssp. <i>paracasei</i> , <i>Lb. fermentum</i> , <i>Lb. brevis</i> , <i>Lb. delbrueckii</i> ssp. <i>delbrueckii</i> , <i>S. thermophilus</i>	79
Pozol	<i>Leuc. mesenteroides</i> , <i>Lb. plantarum</i> , <i>Lb. confusus</i> , <i>Lc. lactis</i> , <i>Lc. raffinolactis</i>	80

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

Adjunct cultures

Secondary cultures, or adjunct cultures or adjuncts, are defined as any cultures that are deliberately added at some point of the manufacture of fermented foods, but whose primary role is not acid production. Adjunct cultures are used in cheese manufacture to balance some of the biodiversity removed by pasteurisation, improved hygiene and the addition of defined-strain starter culture.^{24,25} These are mainly non-starter LAB which have a significant impact on flavour and accelerate the maturation process.^{24,25}

Extracellular polysaccharides (EPSs) are produced by a variety of bacteria and are present as capsular polysaccharides bound to the cell surface, or are released into the growth medium.²⁶ These polymers play a major role in the production of yogurt, cheese, fermented cream and milk-based desserts²⁷ where they contribute to texture, mouth-feel, taste perception and stability of the final products. In addition, it has been suggested that these EPSs or fermented milks containing these EPSs are active as prebiotics,²⁸ cholesterol-lowering²⁹ and immunomodulants.³⁰ EPS-producing strains of *Streptococcus thermophilus* and *Lactobacillus delbreuckii* ssp. *bulgaricus* have been shown to enhance the texture and viscosity of yogurt and to reduce syneresis.³¹

For the production of wine, LAB are involved in the malolactic fermentation, that is a secondary fermentation, which involves the conversion of L-malate to L-lactate and CO₂ via malate decarboxylase, also known as the malolactic enzyme, resulting in a reduction of wine acidity, providing microbiological stabilization and modifications of wine aroma.^{32,33}

Bio-protective cultures

Certain LAB have been found to produce bacteriocins, namely, polypeptides synthesized ribosomally by bacteria that can have a bacteriocidal or bacteriostatic effect on other bacteria.^{34,35} In general, bacteriocins lead to cell death by inhibiting cell wall biosynthesis or by disrupting the membrane through pore formation.³⁶ Bacteriocins are therefore important in food fermentations where they can prevent food spoilage or the inhibition of food pathogens. The best known bacteriocin is nisin, which has gained widespread application in the food industry and is used as a food additive in at least 50 countries, particularly in processed cheese, dairy products and canned foods.³⁷ Examples of useful bacteriocins produced by LAB are lacticin 3147³⁸⁻⁴¹ from *lactococci*, macedovicin from *Streptococcus macedonicus* ACA-DC 198,^{42,43} reuterin from *Lactobacillus reuteri*,⁴⁴ sakacin M from *Lactobacillus sake* 148⁴⁵ curvacin A, curvaticin L442 and lactocin AL705 from *Lactobacillus curvatus* LTH1174,⁴⁶ pediocin PA-1/AcH from *Pediococcus acidilactici*,⁴⁷ plantaricins (A, EF and JK) from *Lactobacillus plantarum*.⁴⁷ The above bacteriocins have proved effective in many food systems for the control of food spoilage or pathogenic bacteria.

Antifungal activities of LAB have been reported.⁴⁸⁻⁵⁰ In addition, LAB strains also have the ability to reduce fungal mycotoxins, either by producing anti-mycotoxinogenic metabolites, or by absorbing them.⁵⁰

For LAB to be used as bio-protective starter cultures, they must possess a range of physical and biochemical characteristics, and most importantly, the ability to achieve growth and sufficient production of antimicrobial metabolites, which must be demonstrated in the specific food environment.⁴⁸

Probiotic cultures

LAB are considered as a major group of probiotic bacteria;^{51,52} probiotic has been defined by Fuller⁵³ as “a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance”. Salminen et al.⁵⁴ proposed that probiotics are microbial cell preparations or components of microbial cells that have a beneficial effect on the health and well-being of the host. Commercial cultures used in food applications include mainly strains of *Lactobacillus* spp., *Bifidobacterium* spp. and *Propionibacterium* spp. *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lb. reuteri*, *Lactobacillus rhamnosus* and *Lb. plantarum* are the most used LAB in functional foods containing probiotics.^{55,56} Argentinean Fresco cheese,⁵⁷ Cheddar⁵⁸ and Gouda⁵⁹ are some examples of applications of probiotic LAB, in combination with bifidobacteria, in cheeses.

The health-promoting effects of LAB are shown in Table 2. Apparently, these effects are species and strain specific, and the big challenge is the use of probiotic cultures composed of multiple species.⁶⁰ In addition, LAB, as part of gut microbiota ferment various substrates such as biogenic amines and allergenic compounds into short-chain fatty acids and other organic acids and gases.⁶¹

Table 2 Effects of probiotics on the human health

Probiotic effect	Reference
Assimilation of cholesterol	79,80
Lactose intolerance	79-81
Control viral, bacterial and antibiotic associated diarrheal diseases	79,80,83-85
Inflammatory bowel disease	79
Allergies and atopic dermatitis	86,87
Colonic carcinogens	88,89
Control of pathogenic bacteria	91,92
Stimulation of the immune system on the gut mucosal surface	93

In recent years, the genomes of several probiotic species have been sequenced, thus paving the way to the application of ‘omics’ technologies to the investigation of probiotic activities.⁶⁰ Moreover, although recombinant probiotics have been constructed, the industrial application of genetically engineered bacteria is still hampered by legal issues and by a rather negative general public opinion in the food sector.⁶⁰

Conclusion

LAB are the most commonly used microorganisms for the fermentation and preservation of foods. Their importance is associated mainly with their safe metabolic activity while growing in foods utilising available sugar for the production of organic acids and other metabolites.

Advances in the genetics, molecular biology, physiology, and biochemistry of LAB have provided new insights and applications for these bacteria. Bacterial cultures with specific traits have been developed during the last 17 years, since the discovery of the complete genome sequence of *Lc. lactis* ssp. *lactis* IL1403 and a variety of commercial starter, functional, bio-protective and probiotic cultures with desirable properties have marketed.

However, the great challenge for food industry is to produce

multiple strain cultures with multiple functions for specific products from specific regions of the world. Also it is a challenge to produce foods, which are similar in sensory characteristics and nutritional value to the traditional products, even with special health-promoting properties, in a standardized, safe and controlled process.

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Conflict of interest

Author declares no conflicts of interest in this paper.

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