LACTIC ACID BACTERIA

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Contents

Summary

The lactic acid bacteria (LAB) may be defined as a group of Gram-positive, nonsporing cocci and rods with nonaerobic habit but aerotolerant, which produce lactic acid as the major end-product during fermentation of carbohydrates. The representative genera of LAB are: *Lactobacillus, Leuconostoc, Pediococcus* and *Streptococcus.* The genus

Bifidobacterium is historically also considered to belong to LAB. Although at present this genus is not included in the group of LAB, due to its significance in the gastrointestinal tract of animals and humans together with some LAB, it will be briefly considered in this article. LAB are subdivided into cocci and rods and into *homofermentative* LAB, which produce mostly lactic acid from sugars and *heterofermentative* LAB which produce in addition to lactic acid considerable amounts of acetic acid and alcohol.

Among fermented foods produced with use of LAB the fermented dairy products are quantitatively the most important. Such products as yoghurt, kefir, sour cream and dips, cultured buttermilk, cheese and many other specialities are produced and consumed worldwide. For sophisticated control, modern industrial processes utilize specially prepared lactic acid bacteria as starter cultures.or „starters„ in the manufacture of fermented dairy products. Lactic acid bacteria play a role in bread-making, particularly rye bread. Some types of sausages are produced using starters of LAB. Products produced in whole or partly by lactic acid fermentation in salt brine are pickles, sauerkraut, and olives. Some species of LAB are components of the normal human intestinal microflora, playing a role in the normal function of the digestive tract and in prevention of intestinal disorders. The growing interest in healthy nutrition stimulated the use of probiotic bacteria (viable bacteria, which have a beneficial effect on the health of animals and humans), particularly of traditionally probiotic strains of LAB and bifidobacteria. Many strains of LAB produce specific compounds with antimicrobial activity, named bacteriocins. Many of these antimicrobial substances are thought to have potential application as preservatives. However, it remains to be determined if these substances will be functional in foods and if they can be produced and function in situ.

1. Introduction

Lactic acid producing fermentation has been known for thousands of years. Many different cultures used this method of food processing to improve the storage quality, palatability, and nutritive value of perishable foods such as milk, meat, fish and some vegetables. The micro-organisms that produce lactic acid are the lactic acid bacteria. In the literature the abbreviation LAB is frequently used. Today lactic acid bacteria are mainly associated with a group of dairy products such as cheese, yoghurt, kefir, and buttermilk. However lactic acid bacteria play an important role in bread production, are are used in technology of some meat products and preservation of vegetables. Lactic acid bacteria have been associated with beneficial health effects. There has been much interest in the use of various strains of lactic acid bacteria as probiotics, i.e. as viable preparations in foods or dietary supplements to improve the health of humans and animals. LAB have been used as probiotics to manage intestinal disorders such as lactose intolerance, acute gastroenteritis, constipation, and inflammatory bowel disease.

The term lactic acid bacteria covers a large group of micro-organisms. The first pure culture of a lactic acid bacterium was obtained in 1873 and the similarity between milk-souring bacteria and other lactic acid-producing bacteria of other habitats was recognized in the early 1900s. The basis of systematic classification of LAB was elaborated and published in 1919 by Orla-Jensen. Although revised to a considerable

extent, the main characteristics of classification have remained unchanged. In the framework of this article, primarily the classification, physiology and industrial use of lactic acid bacteria will be treated. In addition a short review will be given about health aspects of LAB and some future aspects in research and product development on lactic acid bacteria.

2. Classification of Lactic Acid Bacteria

As mentioned above, the general basis of classification of lactic acid bacteria is connected with the work of Orla-Jensen. This classification system at the genus level first divides the LAB, according to morphology, into rods (*Lactobacillus* and *Carnobacterium)* and cocci (all other genera). The next important characteristic used in differentiation of lactic acid bacterium genera is the mode of glucose fermentation under standard conditions (non limited supply with glucose, growth factors such as amino acids, vitamins and nucleic acid precursors, and limited oxygen availability). Under these conditions LAB can be divided into two groups: (I) *homofermentative* and (II) *heterofermentative* bacteria.

Homofermentative LAB convert sugars almost quantitatively to lactic acid. The second group, the heterofermentative bacteria produce not only lactic acid but ethanol/acetic acid, and carbon-dioxide. In practice, a test for gas production from glucose will distinguish between the two groups. Differences were observed also in rate of growth at different temperatures, pH of media, and sodium chloride tolerance. Growth is normally tested at 18 ^{o}C and 45 ^{o}C, sodium chloride concentration of 6.5% and 18%, and pH of 4.4 and 9.3. Finally the formation of different isomeric forms of lactic acid (L-lactic acid or D-lactic acid) may be used to distinguish between different genera. Further classification of species within the genera is very complicated and in many cases even books dealing with microbiology don't give a full overview. To have an idea about the complex nature of such classification, it may be mentioned, for example, that just the genus *Lactobacillus* comprises about 50 recognized species. (Readers interested in details are referred to the work of Wood and Holzapfel included in the Bibliography). Here only a few species important in the food industry will be mentioned. *Streptococcus thermophylus* is used in the manufacture of yogurt. Lactococci, primarily *Lactococcus lactis,* are associated with the dairy industry and the latter is actually used in dairy technology. Species of *Lactobacillus* such as *Lactobacillus acidophylus, Lactobacillus delbrückii, Lactobacillus plantarum,* and *Lactobacillus bulgaricus,* etc. are known in food technology. Details of methods used in differentiation, including some recent trends, will be treated in *Testing Methods in Food Microbiology.*

3. Metabolism of Lactic Acid Bacteria

The main feature of the metabolism of lactic acid bacteria is the degradation of different carbohydrates and related compounds primarily to lactic acid. This is coupled with energy (adenosine-tri-phosphate=ATP) production. Generally the predominant end-product is lactic acid, but changes in growth conditions may result in significantly different end-product patterns. In the following the major fermentation pathways will be shortly reviewed.

3.1. Fermentartion of Hexoses.

From sugars occurring in foods, hexose is most often the substrate for lactic acid bacteria. The end-product of fermentation of glucose under normal conditions (excess sugar and limited access of oxygen) is lactic acid. This means that homofermentative lactic acid bacteria theoretically produce two molecules of lactic acid from one molecule of glucose. Hexoses other than glucose, such as mannose, galactose, and fructose are also fermented by many LAB. These sugars enter the major pathways of glycolysis after isomeration and/or phosphorylation. In further steps of the fermentation process the phosphorylated hexose is split into triose phosphates and then through oxidation and dephosphorylation to pyruvic acid. The final reaction of the fermentation process is the reduction of pyruvic acid to lactic acid. (for details the reader is referred to textbooks in biochemistry). In heterofermentative LAB, another fermentation pathway is typical resulting in two main end-products—lactic acid and ethanol, and also carbon dioxide.

3.2. Disaccharide Fermentation.

Depending on the mode of transport, disaccharides enter the cell either as free sugars or sugar phosphates. In the former case the free disaccharides are split by specific hydrolases to monosaccharides, which then enter the major pathways described above. In the latter case phosphohydrolases split the disaccharide phosphates to 1 mol monosaccharide and 1 mol monosaccharide phosphate. From the point of view of the dairy industry, the fermentation of lactose is the most interesting. Lactose is hydrolysed to its monosaccharide constituents (glucose and galactose); these enter cells either in free form or as phosphate and then the major pathway of lactic acid production. Maltose fermentation among LAB was also studied. This disaccharide is split to two glucoses which may enter the main pathways. Sucrose fermentation is initiated by the cleavage of sucrose hydrolase to glucose and fructose. Both products may enter the main pathways, as shown previously. Fermentation of other disaccharides, such as cellobiose and melibiose, has been poorly studied. The ability of LAB to ferment these sugars differ between the different species. Presumable, the metabolism is mediated by specific transport systems and hydrolases, resulting in the respective monosaccharides, which enter the common pathways.

3.3. Fermentation of pentoses.

Pentoses are readily fermented by lactic acid bacteria. In the cell the pentoses are converted to ribulose-5-phosphate or xilulose-5-phosphate by epimerases and then enter the pentose-phosphate pathway (for details reader are referred to textbooks on biochemistry).

3.4. Other metabolic processes.

In addition to the main metabolic pathways of LAB connected with fermentation of carbohydrates, lactic acid bacteria nitrogen metabolism is connected with hydrolysis and synthesis of proteins. These processes play a significant role in, for example, cheese production and will be treated in the chapter dealing with industrial use of LAB. Lactic acid bacteria may also produce compounds with antimicrobial activity. Finally it should

be noted that LAB have a significant role in creation of flavor of products of dairy industry and baked goods. These aspects will also be briefly discussed in the chapter dealing with industrial use of LAB.

4. Industrial Use of Lactic Acid Bacteria

Preservation of milk by fermentation was used early in human history. Sumerian writings about dairying go back to about 6000 BC. Procedures for the fermentation of meat were developed as early as the fifteenth century BC. in Babylon and China. Methods for the fermentation of vegetables were known in China in the third century BC. Since these times many different cultures in various parts of the world have used LAB to improve storage qualities, palatability, and nutritive value of perishable foods such as milk, meat, fish, and vegetables. Today in the developed world, lactic acid bacteria are mainly associated with fermented dairy products.

4.1. Lactic Acid Bacteria in Dairy Industry

Fermented milks and cheese are dairy products preserved partly by acid produced by bacterial activity. Fermented milks include a lot of products such as yoghurt, acidophilus milk, cultured buttermilk, kefir, taette and various others.

Cultured buttermilk is obtained from pasteurized skim milk or part skim milk cultured with lactic acid and some aroma compound bacteria such as *Streptococcus lactis* and *Streptococcus cremoris*. Sour cream is manufactured by ripening pasteurized cream with lactic acid and aroma producing bacteria. Yoghurt is made from, depending on the type of product, milk with fat content ranging from 1 to 5%. Generally stabilizer is also used in order to produce smoothness, in body and texture, impart gel structure, and reduce wheying or syneresis. Plain yogurt normally contains no added sugar or flavor. Different types of fruit yoghurts are also produced using fruit products as ingredients. *Lactobacillus bulgaricus* and *Streptococcus thermophylus* are the LAB used in such production. Kefir belongs to the class of acid with low alcohol fermented dairy products. It is produced by fermentation with mixed LAB-yeast culture.

Lactic acid fermentation is involved in the making of most kinds of cheese. Cottage cheese is made from pasteurized milk. Coagulation is accomplished with lactic streptococci and rennet. Cottage cheese and other unripened cheese must be chilled and kept cold until consumed. They have a comparatively short keeping time. The Swiss-type cheeses are ripened. During this process, due to activity of lactic acid bacteria (in some types activity of molds) the characteristic texture and aroma will be developed.

The most typical changes during ripening of Swiss-type cheeses are connected with activity of proteolytic enzymes. It is beyond the scope of this article to give even a short overview of production of the more than one hundred types of cheeses produced worldwide. Different peptides, amino acids and, depending on conditions of ripening, amino acids are formed, contributing to the characteristic flavor of cheese. The flavor of cheese is not the result of proteolytic activity only. Lipolysis (primarily in mold-ripened cheese) may also occur and many other compounds connected with the side pathways of lactic acid fermentation are also present.

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Biographical Sketch

Anna Halász D.Sc. is a scientific advisor of Central Research Institute of Food Industry, Budapest, Hungary, and Associate professor of Biochemistry at Budapest University of Technology and Economics. She received her M.Sc. degree from the Technical University (Faculty of Chemical Engineering) in 1961, and her D.Sc. degree in Chemical Sciences in 1988 from the Hungarian Academy of Sciences.Dr Halász is a member of the Food Protein Group of the Hungarian Academy of Sciences, and a member of the Working Group on Yeasts of ICC (International Association for Cereal Science and Technology). She has been the recipient of the Distinguished Researcher Award of the Ministry of Food and Agriculture and she was awarded the Bronze Medal of the Hungarian Republic. She is also a recipient of the Swiss Federal Foundation Fellowship in Science (1970-1971). Dr Halász has published about 200 research papers and is the author of the book Use of Yeast Biomass in Food Production (CRC Press, 1991). Her current major research interests include the biochemistry of yeast and lactic acid bacteria, food quality and safety.