Lactic Acid Bacteria

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Preface

Lactic acid–producing fermentation is an old invention. Many different cultures in various parts of the world have used it to improve the storage qualities, palatability, and nutritive value of perishable foods such as milk, vegetables, meat, fish, legumes, and cereals. The organisms that produce this type of fermentation, lactic acid bacteria, have had an important role in preserving foods, preventing food poisoning, and indirectly feeding the hungry on every continent.

In the developed world, lactic acid bacteria are mainly associated with fermented dairy products such as cheese, buttermilk, and yogurt. The use of dairy starter cultures has become an industry during this century. Because of this, the technological aspects of lactic acid fermentation have been well covered in both research and training in food sciences.

Since the days of the Russian scientist Metchnikoff, lactic acid bacteria have also been associated with beneficial health effects. Today, an increasing number of health foods and so-called functional foods as well as pharmaceutical preparations are promoted with health claims based on the characteristics of certain strains of lactic acid bacteria. Most of these strains, however, have not been thoroughly studied, and consequently the claims are not well substantiated. Moreover, the accepted standards of clinical protocols, including double-blind randomized study designs, have not been applied in most "health-claim" studies—health benefits are judged mainly using subjective criteria. Additionally, the specific bacterial strains used in the studies are often poorly identified. Most information about the health effects of lactic acid bacteria is thus anecdotal. There is a clear need for critical study of the effects on health of strain selection and the quality of fermented foods and their ingredients. Clinical studies should be properly conducted as double-blind, placebo-controlled randomized trials. Both the defined bacterial strains and the proposed products should be studied to verify results. Only such studies produce the solid data that can back up health claims.

This book reviews current developments in the study of lactic acid bacteria using the above-mentioned criteria. An overview of the taxonomy and general physiology of lactic acid bacteria is given. A discussion of the genetics of lactic acid bacteria as a future area of interest is included as well as a chapter on the technological aspects of manufacturing functional lactic acid bacteria starters. Many chapters consider our present knowledge of the effects of lactic acid bacteria in human health and disease and as animal probiotics.

One chapter of particular interest describes the development of individual lactic acid microflora. It was written by an Estonian research group that worked in association with the former Soviet space program. These results have not been previously published in the West.

Thus, this book attempts to shed light on little-known and controversial aspects of lactic acid bacteria and their applications. As new techniques as well as new interest in these organisms develop, the anecdotal evidence on the health benefits of specific strains of lactic acid bacteria is slowly being replaced by a more scientific outlook. This book should serve as an important introduction to any student or scientist interested in these developments.

In particular, those working with lactic acid bacteria and fermented foods or feed products within universities and the food industry should find this book most interesting. It will also be helpful to dairy scientists and technologists, both as a textbook and as a handbook for product development. It will be useful to government organizations developing regulatory policies for products based on lactic acid fermentation and bacteria, especially when health claims are concerned. Finally, consumer groups interested in the effects of lactic acid bacteria may benefit from the comprehensive reviews in this volume.

Readers are referred to most recent literature in the area, covering the subject well from various aspects. Our aim has been to give an overview of a rapidly changing and extremely important area of food and nutrition research.

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Contents

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Lactic Acid Bacteria: Classification and Physiology

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I. SUMMARY

Lactic acid bacteria are a group of Gram-positive bacteria united by a constellation of morphological, metabolic, and physiological characteristics. 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. The boundaries of the group have been subject to some controversy, but there has been general agreement that the genera *Lactobacillus, Leuconostoc, Pediococcus*, and *Streptococcus* form the core of the group. Recent taxonomic revisions of these genera suggest that the lactic acid bacteria comprise the following: *Aerococcus, Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, Streptococcus, Tetragenococcus*, and *Vagococcus*. The classification of lactic acid bacteria into different genera is largely based on morphology, mode of glucose fermentation, growth at different temperatures, configuration of the lactic acid produced, ability to grow at high salt concentrations, and acid or alkaline tolerance. For some of the newly described genera, additional characteristics such as fatty acid composition

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and motility are used in classification. The measurements of true phylogenetic relationships with rRNA sequencing have aided the classification of lactic acid bacteria (LAB) and clarified the phylogeny of the group. Most genera in the group form phylogenetically distinct groups, but some, in particular *Lactobacillus* and *Leuconostoc*, are very heterogeneous and the phylogenetic clusters do not correlate with the current classification based on phenotypic characters. New tools for classification and identification of LAB are underway. The most promising for routine use are nucleic acid probing techniques, partial rRNA gene sequencing using the polymerase chain reaction, and soluble protein patterns.

Two main sugar fermentation pathways can be distinguished among lactic acid bacteria. Glycolysis (Embden-Meyerhof pathway) results in almost exclusively lactic acid as end product under standard conditions, and the metabolism is referred to as homolactic fermentation. The 6-phosphogluconate/phosphoketolase pathway results in significant amounts of other end products, such as ethanol, acetate, and CO_2 in addition to lactic acid, and the metabolism is referred to as heterolactic fermentation. Various growth conditions may significantly alter the end-product formation by some lactic acid bacteria. These changes can be attributed to an altered pyruvate metabolism and/or the use of external electron acceptors such as oxygen or organic compounds.

Lactic acid bacteria creates a proton motive force mainly by means of a membrane-located H^+ATPase at the expense of ATP. The proton motive force drives the uphill transport of metabolites and ions into the cell. End-product efflux may contribute to the formation of a proton motive force, thus sparing ATP. Sugar transport is mediated mainly by proton-motive-force-dependent permease systems or phosphoenolpyruvate–sugar phosphotransferase systems. The latter are tightly coupled to and regulated with sugar metabolism. Transport of amino acids is mediated by proton-motive-force-dependent systems, antiport systems, or phosphate-bond-linked systems.

II. GENERAL INTRODUCTION

What is a lactic acid bacterium? Asking the question to scientists in the field would probably result in a fairly uniform answer. This is due more to historic tradition, dating to the turn of the century, than to the existence of an unequivocal definition of the term. The term *lactic acid bacteria* was then used synonymously with "milk-souring organisms." Important progress in the classification of these bacteria was made when the similarity between milk-souring bacteria and other lactic-acid-producing bacteria of other habitats was recognized (Henneberg, 1904; Löhnis, 1907). However, confusion was still prevalent when the monograph of Orla-Jensen (1919) appeared. This work has had a large impact on the systematics of LAB, and, although revised to some extent, it is still valid and the classification basis is remarkably unchanged. Orla-Jensen used a few characters as classification basis: morphology (cocci or rods, tetrad formation), mode of glucose fermentation (homo- or heterofermentation), growth at certain "cardinal" temperatures (e.g., 10°C and 45°C), and form of lactic acid produced (D, L, or both). As will be seen, these characters are still very important in current LAB classification. After the work by Orla-Jensen, the view emerged that the core of LAB comprised four genera: *Lactobacillus, Leuconostoc, Pediococcus*, and *Streptococcus*. There has always been some controversy on what the boundaries of the group are (Ingram, 1975), but this will not be dealt with here. The classification section of this chapter will concentrate on these four genera, or rather what used to be these genera, since major taxonomic revisions have recently resulted in the description of new genera.

Orla-Jensen regarded LAB as a "great natural group," indicating a belief that the bacteria included were phylogenetically related and separated from other groups. At that time, only phenotypic characters could be examined and evaluated as phylogenetic markers. Today, we have the means to examine, in detail, macromolecules of the cell, believed to be more accurate in defining relationships and phylogenetic positions. These are, of course, the nucleic acids. Fortunately, nature has provided us with different kinds of nucleic acids for different types of taxonomic studies. Close relations (at species and subspecies level) can be determined with DNA-DNA homology studies (Johnson, 1984). For determining phylogenetic positions of species and genera, ribosomal RNA (rRNA) is more suitable, since the sequence contains both well-conserved and less-conserved regions. It is now possible to determine the sequence of long stretches of rRNA (~1500 bases of 16S rRNA) from bacteria (Lane et al., 1985). Comparisons of these sequences are currently the most powerful and accurate technique for determining phylogenetic relationships of microorganisms (Woese, 1987). With this technique, a clearer picture of the phylogeny of LAB is emerging, and the ideas of Orla-Jensen can be examined with some accuracy. In addition, rRNA sequencing is becoming an important aid in the classification of LAB, as exemplified by the descriptions of new genera (Collins et al., 1990; Wallbanks et al., 1990). The classification section of this chapter will deal with both the "classical" classification schemes and the current phylogenetic status of LAB.

The physiology of LAB has been of interest ever since it was recognized that these bacteria are involved in the acidification of food and feed products. Increased knowledge of the LAB physiology, such as metabolism, nutrient utilization, etc., has been one way to achieve more controlled processes. Today, modern genetic techniques are considered to be promising in this regard (see Chapter 6). However, efforts in this direction will not be fruitful unless there is a sound understanding of the physiology of these bacteria. The fermentative nature of LAB is also of considerable academic interest, since this makes them excellent model systems for the study of energy transduction, solute transport, and membrane biology (Kashket, 1987; Konings et al., 1989; Maloney, 1990).

The designation *lactic acid bacteria* perhaps implies that these bacteria have a somewhat "simple" metabolism, resulting in one or a few fermentation end products. This may also be the case in the laboratory environment that we often impose on them. However, it is clear that LAB have a very diverse metabolic capacity, which enables them to adapt to a variety of conditions. The physiology section of this chapter will describe the main features of LAB, such as carbohydrate metabolism and bioenergetics. However, some of the emphasis will be on the different variations of the general "theme" of metabolism that may occur under certain conditions.

This volume concerns the technological, nutritional, and health aspects of LAB. This reflects the intimate association of the term with food and feed manufacture. Again, this is perhaps more of a historic tradition than a scientifically reached position, since the group includes bacteria which are highly pathogenic and therefore undesirable in food (e.g., many streptococci). In addition, lactobacilli generally associated with food have been implicated in disease (Kandler and Weiss, 1986); carnobacteria are normal inhabitants in meat, but are also fish pathogens (Collins et al., 1987). There are more examples of the "dual" nature of LAB as a group. The main emphasis in this chapter will, however, be on LAB that are *normally* associated with food manufacture and positive health aspects.

III. CLASSIFICATION OF LACTIC ACID BACTERIA

A. General Description and Included Genera

An unequivocal definition of the term *lactic acid bacteria* does not exist. Inevitably, most characteristics that would be used in such a definition are subject to qualification (Ingram, 1975), meaning that they are accurate only under conditions that might be termed "normal" or "standard" and that exceptions to the definition can be found. Therefore, it is more appropriate to describe the *typical* lactic acid bacterium, which is Gram-positive, nonsporing, catalase-negative, devoid of cytochromes, of nonaerobic habit but aerotolerant, fastidious, acid-tolerant, and strictly fermentative with lactic acid as the major end product during sugar fermentation. LAB are generally associated with habitats rich in nutrients, such as various food products (milk, meat, vegetables), but some are also members of the normal flora of the mouth, intestine, and vagina of mammals. Variations of this general theme are common, excluding the Gram-positive and nonsporing characters, which cannot be disputed (spore-forming bacteria that resemble LAB, e.g., *Sporolactobacillus*, are more related to bacilli). In any case, the "definition" is useful in being a core or center around which the actual