Biology of Microorganisms on Grapes, in Must and in Wine

Helmut König • Gottfried Unden
Jürgen Fröhlich
Editors

Biology of Microorganisms on Grapes, in Must and in Wine

Springer

Editors:
Professor Dr. Helmut König
Institute of Microbiology
and Wine Research
Johannes Gutenberg-University
Becherweg 15
55099 Mainz
Germany
hkoenig@uni-mainz.de

Professor Dr. Gottfried Unden
Institute of Microbiology
and Wine Research
Johannes Gutenberg-University
Becherweg 15
55099 Mainz
Germany
unden@uni-mainz.de

Dr. Jürgen Fröhlich
Institute of Microbiology
and Wine Research
Johannes Gutenberg-University
Becherweg 15
55099 Mainz
Germany
jfroehl@uni-mainz.de

Cover illustration top: Sporangiophore with sporangia from *Plasmopara viticola*; Low-Temperature-Scanning-Electron-Microscopy (H.-H. Kassemeyer, State Institute for Viticulture and Oenology, Freiburg; S. Boso and M. Düggelin, University of Basel); *below*: Microscope image of a mixture of *Dekkera/Brettanomyces* yeast species (Christoph Röder, Institute of Microbiology and Wine Research, University of Mainz)

ISBN: 978-3-540-85462-3 e-ISBN: 978-3-540-85463-0
DOI: 10.1007/978-3-540-85463-0

Library of Congress Control Number: 2008933506

Cover design: WMX Design GmbH, Heidelberg, Germany

Printed on acid-free paper

9 8 7 6 5 4 3 2 1

springer.com

Foreword

The ancient beverage wine is the result of the fermentation of grape must. This naturally and fairly stable product has been and is being used by many human societies as a common or enjoyable beverage, as an important means to improve the quality of drinking water in historical times, as therapeutical agent, and as a religious symbol.

During the last centuries, wine has become an object of scientific interest. In this respect different periods may be observed. At first, simple observations were recorded, and subsequently, the chemical basis and the involvement of microorganisms were elucidated. At a later stage, the scientific work led to the analysis of the many minor and trace compounds in wine, the detection and understanding of the biochemical reactions and processes, the diversity of microorganisms involved, and the range of their various activities. In recent years, the focus shifted to the genetic basis of the microorganisms and the molecular aspects of the cells, including metabolism, membrane transport, and regulation. These different stages of wine research were determined by the scientific methods that were known and available at the respective time.

The recent "molecular" approach is based on the analysis of the genetic code and has led to significant results that were not even imaginable a few decades ago. This new wealth of information is being presented in the Biology of Microorganisms on Grapes, in Must, and in Wine. The editors were lucky in obtaining the cooperation of many specialists in the various fields. This joint international effort has resulted in a comprehensive book presenting our present day knowledge of a specialized group of organisms that are adapted to the very selective habitat of wine. The various contributions of the book have the character of reviews and contain an extensive bibliography, mainly of the actual scientific papers.

I sincerely wish the editors and the authors that the presented book will be widely received by the scientific community and will be frequently used as a welcome source of information and a helpful means for further work on the microorganisms of wine. Furthermore, understanding the intricate microbiological and biochemical processes during the fermentation should be helpful in the production of wine.

Mainz, June 2008 Ferdinand Radler

Preface

"Ce sont les microbes qui ont le dernier mot"
(Louis Pasteur)

Archaeology, genetics, ancient literature studies (*Epic of Gilgamesh*, ca. 2000 BC), paleobotany and linguistics point to the Neolithic period (ca. 8000 BC) as the time when domestic grape growing (*Vitis vinifera vinifera*) and wine making began, most probably in Transcaucasia (P. E. McGovern, 2003). For ages wine has been an essential part of the gracious, cultured and religious way of life.

Starting at the heartlands of Middle East, winemaking techniques have been empirically improved since neolithic times, expanding into experimental and scientific viticulture and oenology in our days. Despite these long traditions in wine making it was only 1857 that significant contributions of Louis Pasteur on alcoholic and lactic acid fermentation, as well as on acetic acid formation, proved that the conversion of grape juice into wine was a microbiological and not a purely chemical process.

Up to now, bounteous knowledge about wine making techniques and procedures has been accumulated, which was already found in several books about wine microbiology, biotechnology and laboratory practices. Especially in the last two decades, our knowledge about the role of microbes and their application as starter culture has been greatly increased.

Therefore, the aim of this book is to focus on the ecological and biological aspects of the wine-associated microbiota, starting from grape-colonising to wine-spoiling microbes. Purely technical aspects of winemaking are not a subject of this publication.

Growth in the must and wine habitat is limited by low pH values and high ethanol concentrations. Therefore, only acid- and ethanol-tolerant microbial groups can grow in grape juice, must and wine, which include lactic acid and acetic acid bacteria, yeasts and fungi. The most important species for wine-making are *Saccharomyces cerevisiae* and *Oenococcus oeni*, which perform the ethanol and malolactic fermentation, respectively. These two species are also applied as starter cultures. However, the diverse other microorganisms growing on grapes and must have a significant influence on wine quality.

The book begins with the description of the diversity of wine-related microorganisms, followed by an outline of their primary and energy metabolism. Subsequently, important aspects of the secondary metabolism are dealt with, since these activities have an impact on wine quality and off-flavour formation. Then chapters about stimulating and inhibitory growth factors follow. This knowledge is helpful for the growth management of different microbial species. During the last twenty years, significant developments have been made in the application of the consolidated findings of molecular biology for the rapid and real-time identification of certain species in mixed microbial populations of must. Basic knowledge was acquired about the functioning of regulatory cellular networks, leading to a better understanding of the phenotypic behaviour of the microbes in general and especially of the starter cultures as well as of stimulatory and inhibitory cell-cell interactions during winemaking. In the last part of the book, a compilation of some modern methods round off the chapters.

This broad range of topics about the biology of the microbes involved in the vinification process could be provided in one book only because of the input of many experts from different wine-growing countries. We thank all the authors for offering their experience and contributions. Finally, we express our special thanks to Springer for agreeing to publish this book about wine microbes.

We hope that this publication will help winemakers as well as scientists and students of oenology to improve their understanding of microbial processes during the conversion of must to wine.

Mainz
June 2008

Helmut König
Gottfried Unden
Jürgen Fröhlich

Contents

Contributors

M. Teresa Alegre
Departamento de Microbiología y Parasitología, Escuela Politécnica Superior, Universidad de Santiago de Compostela, Campus Universitario, 27002-Lugo, Spain
mtalegre@lugo.usc.es

Eveline J. Bartowsky
The Australian Wine Research Institute, P. O. Box 197, Glen Osmond, Adelaide, SA 5064, Australia
Eveline.Bartowsky@awri.com.au

Beate Berkelmann-Löhnertz
Institut für Biologie, Forschungsanstalt Geisenheim, 65366 Geisenheim, Germany
berkelmann@fa-gm.de

Linda F. Bisson
Department of Viticulture and Enology, University of California, Davis, CA 95616, USA
lfbisson@ucdavis.edu

Bruno Blondin
Unité d'Oenologie Agro.M, IHEV, UMR Sciences pour l'Oenologie INRA, Equipe Microbiologie, 2, place viala, 34060 Montpellier Cedex, France
blondin@supagro.inra.fr

Anthony R. Borneman
The Australian Wine Research Institute, P. O. Box 197, Glen Osmond, Adelaide, SA 5064, Australia
Anthony.Borneman@awri.com.au

Paul J. Chambers
The Australian Wine Research Institute, P. O. Box 197, Glen Osmond, Adelaide, SA 5064, Australia
Paul.Chambers@awri.com.au

Harald Claus
Institute of Microbiology and Wine Research, Johannes Gutenberg-University, 55099 Mainz, Germany
hclaus@uni-mainz.de

Marcel·lí del Olmo
Departament de Bioquímica i Biologia Molecular, Facultad de Biológicas, Universitat de València, Dr. Moliner 50, E46100 Burjassot, Spain

Sylvie Dequin
UMR 1083 Sciences pour l'Oenologie INRA, Montpellier SupAgro, UM1, Equipe Microbiologie, 2 place Viala, 34060 Montpellier Cedex, France
dequin@inra.ensam.fr

Nicolas Desroche
Nexidia SAS, 26 Bd Petitjean BP 8999, 21079 Dijon, France
nicolas.desroche@nexida.fr

Leon Milner Theodore Dicks
Department of Microbiology, University of Stellenbosch, 7600 Stellenbosch, South Africa
LMTD@sun.ac.za

Helmut Dietrich
Geisenheim Research Center, Section Wine Analysis and Beverage Technology, Rüdesheimer Str. 28, D-65366 Geisenheim, Germany
H.Dietrich@fa-gm.de

Marguerite Dols-Lafargue
UMR 1219 Œnologie, Université Victor Segalen Bordeaux 2, INRA, ISVV, 351 cours de la Libération, 33405 Talence, France
m.dols@istab.u-bordeaux1.fr

Armin Ehrenreich
Lehrstuhl für Mikrobiologie, Technische Universität München, Am Hochanger 4, 85354 Freising, Germany
aehrenr@mikro.biologie.tu-muenchen.de

Akihito Endo
Department of Microbiology, University of Stellenbosch, 7600 Stellenbosch, South Africa
aki@sun.ac.za

Jürgen Fröhlich
Erbslöh Geisenheim AG, Erbslöhstraße 1, 65366 Geisenheim, Germany
juergen.froehlich@erbsloeh.com

José García-Martínez
Sección de Chips de DNA-S.C.S.I.E., Universitat de València, Dr. Moliner 50, E46100 Burjassot, Spain
Jose.Garcia-Martinez@uv.es

Lisa Granchi
Department of Agricultural Biotechnology, Section of Microbiology, University of Florence, P.le delle Cascine, 24, 50144 Firenze, Italy
lisa@granchi@unifi.it

Simona Guerrini
Department of Agricultural Biotechnology, Section of Microbiology, University of Florence, P.le delle Cascine, 24, 50144 Firenze, Italy
simona.guerrini@unifi.it

José Manuel Guillamón
Departamento de Biotecnología. Instituto de Agroquímica y Tecnología de los Alimentos (CSIC), Apartado de Correos 73, 46100-Burjasot (Valencia) Spain
guillamon@iata.csic.es

Jean Guzzo
Equipe de Recherche en Vigne et Vin, Institut Jules Guyot, Rue Claude Ladrey BP 27877, 21078 Dijon, France
jguzzo@u-bourgogne.fr

Jürgen J. Heinisch
Universität Osnabrück, Fachbereich Biologie/Chemie, AG Genetik, Barbarastr. 11, 49076 Osnabrück, Germany
heinisch@biologie.uni-osnabrueck.de

C.M. Lucy Joseph
Department of Viticulture and Enology, University of California, Davis, Davis, CA 95616, USA
cmjoseph@ucdavis.edu

Hanns-Heinz Kassemeyer
Department for Biology, State Institute for Viticulture and Oenology, 79100 Freiburg, Germany
hanns-heinz.Kassemeyer@wbi.bwl.de

Helmut König
Institute of Microbiology and Wine Research, Johannes Gutenberg-University, 55099 Mainz, Germany
hkoenig@uni-mainz.de

Sibylle Krieger-Weber
Lallemand Inc., In den Seiten 53, 70825 Korntal-Münchingen, Germany
skrieger@lallemand.com

Jean-Luc Legras
UMR Santé de la Vigne et Qualité du Vin INRA, Université Louis Pasteur Strasbourg, 28, rue de Herrlisheim, BP 20507, 68021 Colmar Cedex, France
legras@colmar.inra.fr

Aline Lonvaud-Funel
UMR 1219 Œnologie, Université Victor Segalen Bordeaux 2, INRA, ISVV,
351 cours de la Libération, 33405 Talence, France
aline.lonvaud@oenologie.u-bordeaux2.fr

Silvia Mangani
Department of Agricultural Biotechnology, Section of Microbiology,
University of Florence, P.le delle Cascine, 24, 50144 Firenze, Italy
silvia.mangani@unifi.it

Angela M. Marcobal
Department of Viticulture and Enology, University of California at Davis,
One Shields Avenue, Davis, CA 95616, USA
amarcobal@ucdavis.edu

Albert Mas
Biotecnologia Enològica, Departament de Bioquímica i Biotecnologia,
Facultat de Enologia, Universitat Rovira i Virgili. Marcelćli Domingo s/n,
43007, Tarragona, Spain
albert.mas@urv.cat

Juan M. Mesas
Departamento de Química Analítica Nutrición y Bromatología
(Área de Tecnología de Alimentos), Escuela Politécnica Superior,
Universidad de Santiago de Compostela, Campus Universitario,
27002-Lugo, Spain
jmesas@lugo.usc.es

David A. Mills
Department of Viticulture and Enology, University of California at Davis,
One Shields Ave., Davis, CA 95616, USA
damills@ucdavis.edu

José E. Pérez-Ortín
Departament de Bioquímica i Biologia Molecular, Facultad de Biológicas,
Universitat de València, Dr. Moliner 50, E46100 Burjassot, Spain
jose.e.perez@uv.es

Peter Pfeiffer
Institute of Microbiology and Wine Research, Johannes Gutenberg-University,
55099 Mainz, Germany
ppfeiffe@uni-mainz.de

Martin Shahin Pour-Nikfardjam
Staatl. Lehr- und Versuchsanstalt für Wein- und Obstbau D-74189
Weinsberg, Germany
martin.pourin@wwo.lvwl.de

Isak S. Pretorius
The Australian Wine Research Institute, P. O. Box 197, Glen Osmond, Adelaide, SA 5064, Australia
Sakkie.Pretorius@awri.com.au

Amparo Querol
Departamento de Biotecnología, Instituto de Agroquímica y Tecnología de Alimentos (CSIC), P.O. Box 73, 46100 Burjassot, València, Spain
aquerol@iata.csic.es

Doris Rauhut
Fachgebiet Microbiologie und Biochemie, Forschungsanstalt Geisenheim, Von-Lade-Straße 1, D-65366, Geisenheim
doris.rauhut@fa-gm.de

Ferdinand Radler
Institute of Microbiology and Wine Research,
Johannes Gutenberg-Universität, Becherweg 15, D-5509 Mainz, Germay
f.radler@arcor.de

Rosaura Rodicio
Departamento de Bioquímica y Biología Molecular and Instituto Universitario de Biotecnología de Asturias, Universidad de Oviedo, 33006 Oviedo, Spain
rosaura@sauron.quimica.uniovi.es

Mário A. Santos
Instituto de Ciência Aplicada e Tecnologia, Departamento de Biologia Vegetal, Faculdade de Ciências da Universidade de Lisboa. Campo Grande, 1749-016 Lisboa, Portugal
mmsantos@fc.ul.pt

Carlos São-José
Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa Av. Prof. Egas Moniz, Ed. Egas Moniz, 1649-028 Lisboa, Portugal
cjose@fc.ul.pt

Manfred J. Schmitt
Angewandte Molekularbiologie, FR 8.3 Biowissenschaften, Universität des Saarlandes, Campus Gebäude A 1.5, 66041 Saarbrücken, Germany
mjs@microbiol.uni-sb.de

S.D. Todorov
Department of Microbiology, University of Stellenbosch, 7600 Stellenbosch, South Africa
todorov@sun.ac.za

Gottfried Unden
Institut für Mikrobiologie und Weinforschung, Johannes Gutenberg-Universität Mainz, Becherweg 15, 55099 Mainz, Germany
unden@uni-mainz.de

Massimo Vincenzini
Department of Agricultural Biotechnology, Section of Microbiology,
University of Florence, P.le delle Cascine, 24, 50144 Firenze, Italy
massimo.vincenzini@unifi.it

Tanja Zaunmüller
Institut für Mikrobiologie und Weinforschung, Johannes Gutenberg-Universität Mainz
Becherweg 15, 55099 Mainz, Germany
zaunmuel@uni-mainz.de

Part I
Diversity of Microorganisms

Chapter 1
Lactic Acid Bacteria

Helmut König and Jürgen Fröhlich

1.1 Introduction

In 1873, ten years after L. Pasteur studied lactic acid fermentation (between 1857 and 1863), the first pure culture of a lactic acid bacterium (LAB) ("*Bacterium lactis*") was obtained by J. Lister. Starter cultures for cheese and sour milk production were introduced in 1890, while fermented food has been used by man for more than 5,000 years (Schlegel 1999; Stiles and Holzapfel 1997). The first monograph by S. Orla-Jensen appeared in 1919. A typical lactic acid bacterium grown under standard conditions (nonlimiting glucose concentration, growth factors and oxygen limitation) is gram-positive, nonsporing, catalase negative in the absence of porphorinoids, aerotolerant, acid tolerant, organotrophic, and a strictly fermentative rod or coccus, producing lactic acid as a major end product. It lacks cytochromes and is unable to synthesize porphyrins. Its features can vary under certain conditions. Catalase and cytochromes may be formed in the presence of hemes and lactic acid can be further metabolized, resulting in lower lactic acid concentrations. Cell division occurs in one plane, except pediococci. The cells are usually nonmotile. They have a requirement for complex growth factors such as vitamins and amino acids. An unequivocal definition of LAB is not possible (Axelsson 2004).

Lactic acid bacteria are characterized by the production of lactic acid as a major catabolic end product from glucose. Some bacilli, such as *Actinomyces israeli* and bifidobacteria, can form lactic acid as a major end product, but these bacteria have rarely or never been isolated from must and wine. The DNA of LAB has a G + C content below 55 mol%. LAB are grouped into the *Clostridium* branch of gram-positive bacteria possessing a relationship to the bacilli, while *Bifidobacterium* belongs to the Actinomycetes. They are grouped in one order and six families. From the 32 described genera, only 22 species belonging to five genera have been isolated from must and wine (Table 1.1).

H. König (✉)
Institute of Microbiology and Wine Research, Johannes Gutenberg-University,
55099 Mainz, Germany
hkoenig@uni-mainz.de

H. König et al. (eds.), *Biology of Microorganisms on Grapes, in Must and in Wine*,

The homofermentative species produce lactic acid (<85%) as the sole end product, while the heterofermentative species produce lactic acid, CO_2 and ethanol/acetate. At least half of the end product carbon is lactate. Heterofermentative LAB utilizes the pentose phosphate pathway, alternatively referred to as the phosphoketolase or phosphogluconate pathway. Homofermentative wine-related LAB include pediococci and group I lactobacilli. Obligate heterofermentative wine-related LAB include *Leuconostoc*, *Oenococcus*, *Weissella* and group III lactobacilli (Tables 1.2–1.5).

Table 1.1 Current taxonomic outline of lactic acid bacteria[a] of the *Clostridium* branch

Phylum Class Order	Family	Genus	Species from Must and Wine
"Firmicutes" "Bacilli" "Lactobacillales"	I. Lactobacillaceae	I. *Lactobacillus*	*Lb. brevis*, *Lb. buchneri*, *Lb. casei*, *Lb. curvatus*, *Lb. delbrueckii*, *Lb. diolivorans*, *Lb. fermentum*, *Lb. fructivorans*, *Lb. hilgardii*, *Lb. jensenii*, *Lb. kunkeei*, *Lb. mali*, *Lb. nagelii*, *Lb. paracasei*, *Lb. plantarum*, *Lb. vini*
		II. *Paralactobacillus*	
		III. *Pediococcus*	*P. pentosaceus*, *P. parvulus*, *P. damnosus*
	II. "Aerococcaceae"	I. *Aerococcus*	
		II. *Abiotrophia*	
		III. *Dolosicoccus*	
		IV. *Eremococcus*	
		V. *Facklamia*	
		VI. *Globicatella*	
		VII. *Ignavigranum*	
	III. "Carnobacteriaceae"	I. *Carnobacterium*	
		II. *Agitococcus*	
		III. *Alkalibacterium*	
		IV. *Allofustis*	
		V. *Alloiococcus*	
		VI. *Desemzia*	
		VII. *Dolosigranulum*	
		VIII. *Granulicatella*	
		IX. *Isobaculum*	
		X. *Lactosphaera*	
		XI. *Marinilactibacillus*	
		XII. *Trichococcus*	

(continued)

Table 1.1 (continued)

Phylum Class Order	Family	Genus	Species from Must and Wine
	IV. "Enterococcaceae"	I. *Enterococcus*	
		II. *Atopobacter*	
		III. *Melissococcus*	
		IV. *Tetragenococcus*	
		V. *Vagococcus*	
	V. "Leuconostocaceae"	I. *Leuconostoc*	*Lc. mesenteroides*
		II. *Oenococcus*	*O. oeni*
		III. *Weissella*	*W. paramesenteroides*
	VI. Streptococcaceae	I. *Streptococcus*	
		II. *Lactococcus*	

[a]Garrity GM (2005). Principal genera of LAB are underlined (Axelsson 2004)

Table 1.2 Differential characteristics of the wine-related lactic acid genera

Genus	Morphology from Glc	Carbohydrate fermentation[a]	Lactic acid isomer
Lactobacillus	Rods, coccobacilli cells single or in chains	homo- or heterofermentative facultatively heterofermentative	D, L, DL
Leuconostoc[b]	Spherical or lenticular cells in pairs or chains	heterofermentative	D
Oenococcus[b]	Spherical or lenticular cells in pairs or chains	heterofermentative	D
Pediococcus	Spherical cells, pairs or tetrads	homofermentative or facultatively heterofermentative[c]	DL, L
Weissella	Spherical, lenticular, irregular cells	heterofermentative	D, DL

[a]nonlimiting concentration of glucose and growth factors, but oxygen limitation.
[b]Differentiation of wine-related species of *Leuconostoc* and *Oenococcus* cf. Table 1.4.
[c]Facultatively heterofermentative species: *P. pentosaceus, P. acidilactici, P. claussenii.*

Our present knowledge about LAB in general (Carr et al. 1975; Wood and Holzapfel 1995; Holzapfel and Wood 1998; Wood 1999; Wood and Warner 2003; Salminen et al. 2004) and their activities on grape or in must and wine (Fleet 1993; Dittrich and Großmann 2005; Ribéreau-Gayon et al. 2006a, b; Fugelsang and Edwards 2007) has been compiled in several books.

1.2 Ecology

In general, LAB occur in habitats with a rich nutrition supply. They occur on decomposing plant material and fruits, in dairy products, fermented meat and fish, beets, potatoes, mash, sauerkraut, sourdough, pickled vegetables, silage, beverages, plants, water, juices, sewage and in cavities (mouth, genital, intestinal and respiratory tract) of human and animals. They are part of the healthy microbiota of the

human gut. Apart from dental caries, lactobacilli are generally considered apathogenic. *Lb. plantarum* could be associated with endocarditis, septicemia and abscesses. Some species are applied as starter cultures for food fermentation. Because of the acidification they prevent food spoilage and growth of pathogenic microorganisms (Hammes et al. 1991). Some LAB are employed as probiotics, which are potentially beneficial bacterial cells to the gut ecosystem of humans and other animals (Tannock 2005).

Lactic acid bacteria can also be found on grapes, in grape must and wine, and beer. Undamaged grapes contain $<10^3$ CFU per g and the initial titer in must is low (Lafon-Lafourcade et al. 1983). Because of the acidic conditions (pH: 3.0–3.5) grape must provides a suitable natural habitat only for a few microbial groups which are acid tolerant such as LAB, acetic acid bacteria and yeasts. While many microbes are inhibited by ethanol concentrations above 4 vol%, ethanol tolerant species survive in young wine or wine. Besides yeasts, some *Lactobacillus* species (e.g. *Lb. hilgardii*) and *Oenococcus oeni* can grow at higher ethanol concentrations. While only a few LAB species of the genera *Lactobacillus* (*Lb.*), *Leuconostoc* (*Lc.*), *Pediococcus* (*P.*), *Oenococcus* (*O.*) and *Weissella* (*W.*) (Table 1.1 and 1.2) and the acetic acid genera *Acetobacter* and *Gluconobacter* can grow in must and wine, more than 90 yeast species have been found. Malolactic fermentation by lactic acid bacteria is occasionally desirable during vinification, but they can also produce several off-flavours in wine. The genera *Carnobacterium*, *Enterococcus*, *Lactococcus*, *Streptococcus* and *Bifidobacterium* have not been isolated from must and wine.

1.3 Phenotypic and Phylogenetic Relationship

The classification of LAB is largely based on morphology (rods, cocci, tetrads), mode of glucose fermentation, substrate spectrum, growth at different temperatures (15 and 45°C), configuration of lactic acid produced, ability to grow at high salt concentrations (6.5% NaCl; 18% NaCl), and acid, alkaline or ethanol tolerance, as well as fatty acid composition and cell wall composition, lactic acid isomers from glucose, behaviour against oxygen (anaerobic or microaerophilic growth), arginine hydrolysis, acetoin formation, bile tolerance, type of hemolysis, production of extracellular polysaccharides, growth factor requirement, presence of certain enzymes, growth characteristics in milk, serological typing, murein, teichoic acid and menaquinone type, fatty acid composition and electrophoretic mobility of the lactate dehydrogenases and DNA, PCR-based fingerprinting techniques, DNA-DNA homology and soluble protein pattern, 16S rDNA and gene sequencing (e.g. *recA*) (Axelsson 2004).

The genera and species of lactic acid bacteria occurring in must and wine can be differentiated by phenotypic features (Tables 1.2–1.5). The species can be identified by the API 50 CHL identification system (Bio-Mérieux) or the Biolog Microbial Identification System (Biolog, Inc.).

The first taxonomic outline given by Orla-Jensen (1919) is still of some importance. Based on physiological features Kandler and Weiss (1986) divided the genus *Lactobacillus* into the three groups (1) obligate homofermenters, (2) faculative heterofermenters and (3) obligate heterofermenters (Table 1.3). The phylogenetic relationship has been revealed by rRNA sequencing (Fig. 1; Collins et al. 1990, 1991,1993; Martinez-Murcia and Collins 1990; Dicks et al. 1995). According to the 16S rDNA analysis Collins et al. (1990, 1991, 1993) divided the genus *Lactobacillus* into three groups. Group I contains obligate homofermentative species and facultatively heterofermentative species. Group II contains more than 30 *Lactobacillus* species and five pediococcal species. The wine-related facultative heterofermenters *Lb. casei* and the obligate heterofermenters *Lb. brevis*, *Lb. buchneri* and *Lb. fermentum* belong to this group. Group III contains the genus *Weissella,* the leuconostocs (*Lc. mesenteroides)* and *O. oeni*. Schleifer and Ludwig (1995a, b) proposed the phylogenetic groups (1) *Lb. acidophilus* group, (2) *Lb. salivarius* group, (3) *Lb. reuteri* group (*Lb. fermentum*), (4) *Lb. buchneri* group (*Lb. buchneri*, *Lb. fructovorans*, *Lb. hilgardii*) and (5) *Lb. plantarum* group.

The *Leuconostoc* group can be clearly separated from other lactobacilli (Collins et al. 1991; Schleifer and Ludwig 1995a, b). The wine-related species *Lc. mesenteroides* forms a subgroup of the obligately heterofermentative *Leuconostoc* group. *Lc. oenos* was placed in the separate genus *Oenococcus* (Dicks et al. 1995) consisting of the two species *O. oeni* and *O. kitahareae* (Endo and Okada 2006). The latter was isolated from a composting distilled shochu residue. It does not grow at acidic conditions (pH 3.0–3.5) of must and lacks the ability to perform malic acid degradation.

Hammes and Hertel (2003) described seven phylogenetic groups, which were modified by Dellaglio and Felis (2005) (cf. Table 1.3).

1.4 Physiology

Carbohydrates are used as carbon and energy source by a homofermentative or heterofermentative pathway. Sugars or oligosaccharides are taken up by the phosphotransferase system (PTS, e.g. lactose: *Lb. casei*) or the permease system. Homofermentation of hexoses procedes via the Embden-Meyerhof-Parnas pathway, while heterofermentation is performed via the 6-P-gluconate/phosphoketolase pathway resulting in lactate, acetate/ethanol and CO_2 as endproducts or the Bifidus pathway (*Bifidobacterium*). Pentoses are fermented by 6-phosphocluconate/phosphoketolase pathway leading to lactic acid, acetic acid/ethanol and carbon dioxide. Some lactibacilli such as *Lb. salivarius* (Raibaud et al. 1973) or *Lb. vini* (Rodas et al. 2006) can ferment pentoses homofermentatively. Some strains can produce acetate, ethanol and formate from pyruvate under low substrate concentrations and strictly anaerobic conditions (Hammes and Vogel 1995). Lactic acid bacteria form D(−) or L(+) lactic acid or a racemic mixture of lactic acid isomers (Kandler 1983).

Table 1.3 Differential characteristics of wine-related species of the genus *Lactobacillus*

Characteristics	*Lb. brevis*	*Lb. buchneri*	*Lb. casei*[a]	*Lb. curvatus*	*Lb. delbrueckii*[d]	*Lb. diolivorans*	*Lb. fermentum*	*Lb. fructivorans*[b]
Phylogenetic group	I	A	D	G	C	A	F	A
Fermentation mode	III	III	II	II	I	III	III	III
Mol% G + C	44–47	44–46	45–47	42–44	49–51	40	52–54	38–41
Murein type	Lys-D-Asp	Lys-D-Asp	Lys-D-Asp	Lys-D-Asp	Lys-D-Asp	n.d.	Orn-D-Asp	Lys-D-Asp
Teichoic acid	glycerol	n.d.	n.d.	n.d.	n.d.	n.d.	ribitol or	n.d.
Lactic acid	DL	DL	L	DL	D	n.d.	DL	DL
Growth at 15/45 °C	+/–	+/–	+/–	+/-	–/+	+/–	–/+	+/–
NH_3 from Arg	+	+	n.d.	n.d.	d	n.d.	+	+
Fermentation of:								
Amygdalin	n.d.	n.d.	+	–	+	–	n.d.	n.d.
L-Arabinose	+	+	–	–	n.d.	+	d	–
Cellobiose	–	–	+	+	d	–	d	–
Esculin	d	d	+	+	n.d.	n.d.	–	–
Galactose	d	d	n.d.	n.d.	d	+	+	–
Gluconate	n.d.	n.d.	+	+	n.d.	+	n.d.	n.d.
Lactose	n.d.	n.d.	n.d.	n.d.	+	–	n.d.	n.d.
Maltose	+	+	n.d.	n.d.	+	+	+	d
Mannitol	n.d.	n.d.	+	+	–	–	n.d.	n.d.
D-Mannose	–	–	n.d.	n.d.	+	–	w	–
Melezitose	–	+	+	–	n.d.	–	–	–
Melibiose	+	+	–	–	–	+	+	–
D-Raffinose	d	d	–	–	–	w	+	–
Ribose	+	+	+	–	n.d.	+	+	w
Salicin	n.d.	n.d.	n.d.	n.d.	+	–		n.d.
Sorbitol	n.d.	n.d.	+	–	n.d.	–	n.d.	n.d.
Sucrose	d	d	+	+	+	–	+	d
Trehalose	–	–	n.d.	n.d.	+	n.d.	d	–
D-Xylose	d	d	–	–	n.d.	+	d	–

Characteristics	*Lb. hilgardii*[b]	*Lb. jensenii*	*Lb. kunkeei*	*Lb. mali*	*Lb. nagelii*	Lb. paracasei[e]	*Lb. plantarum*[c]	*Lb. vini*
Phylogenetic group	A	C	B	H	H	D	E	H
Fermentation mode	III	II	III	I	I	II	II	I
Mol% G + C	39–41	35–37	n.d.	32–34	n.d.	45–47	44–46	39
Murein type	Lys-D-Asp	Lys-D-Asp	Lys-D-Asp	mDAP direct	mDAP direct	Lys-D-Asp	mDAP direct	Lys-D-Asp
Teichoic acid glycerol	glycerol	n.d.	n.d.	n.d.	n.d.	n.d.	ribitol or	n.d.
Lactic acid	DL	D	L	L	DL	L	DL	DL
Gowth at 15/45 °C	+/–	–/+	+/–	+/ n.d.	+/+	+/n.d.	–/+	–/+
NH_3 from Arg	+	+	+	n.d.	–	n.d.	–	–
Fermentation of:								
Amygdalin	n.d.	+	–	n.d.	+	+	+	+
Arabinose	–	n.d.	–	–	–	–	d	+
Cellobiose	–	+	–	+	+	+	+	+
Esculin	–	n.d.	–	n.d.	n.d.	+	+	+
Galactose	d	+	–	n.d.	+	n.d.	n.d.	–
Gluconate	n.d.	n.d.	–	n.d.	–	+	+	–
Lactose	n.d.	–	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Maltose	+	d	–	–	+	n.d.	n.d.	+
Mannitol	n.d.	d	+	+	+	+	+	–
D-Mannose	–	+	–	n.d.	+	n.d.	n.d.	+
Melezitose	d	–	–	n.d.	–	+	+	–
Melibiose	–	n.d.	–	n.d.	–	–	+	–
D-Raffinose	–	–	w	n.d.	–	–	+	–
Ribose	+	n.d.	–	n.d.	–	+	+	+
Salicin	n.d.	+	n.d.	n.d.	+	n.d.	n.d.	n.d.
Sorbitol	n.d.	n.d.	–	+	+	d	+	–
Sucrose	d	+	+	n.d.	+	+	+	+
Trehalose	–	+	–	n.d.	+	n.d.	n.d.	+
D-Xylose	+	n.d.	–	n.d.	–	–	d	–

+, ≥90% of the strains are positive; –, ≥90% of the strains are negative; *d* 11–89% of the strains are positive; *w* weak positive reaction (Hammes and Vogel 1995). Three phylogentic groups (Hammes and Vogel 1995; Schleifer and Ludwig 1995a, b) were described in 1995 (group A: *Lb. delbrueckii* group; group B: *Lb. casei-Pediococcus* group; group C: *Leuconostoc* group). Eight years later Hammes and Hertel (2003) described seven phylogenetic groups, which were modified by Dellaglio and Felis (2006) (wine-related species are given in brackets): A. *Lb. buchneri* group (group a: *Lb. buchneri*, *Lb. diolivorans*, *Lb. hilgardii*; group b: *Lb. fructivorans*). B. *Lb. kunkeei* group (*Lb. kunkeei*). C. *Lb. delbrueckii* group (*Lb. delbruechii*, *Lb. jensenii*). D. *Lb. casei* group (group a: *Lb. casei*, *Lb. paracasei*). E. *Lb. plantarum* group (group a: *Lb. plantarum*). F. *Lb. reuteri* group (group a: *Lb. fermentum*). G. *Lb. sakei* group (*Lb. curvatus*). H. *Lb. salivarius* group (*Lb. mali*, *Lb. nagelii, Lb. vini*). I. *Lb. brevis* group (*Lb. brevis*). Definition of the fermentative groups (Kandler and Weiss 1986; Hammes and Vogel 1995; Schleifer and Ludwig 1995a, b): Group I: Obligately homofermentative lactobacilli. Hexoses are almost exclusively (>85%) fermented to lactic acid by

(continued)

Table 1.3 (continued)

the Embden-Meyerhof-Parnas pathway (EMP). The organisms possess a fructose-1.6-bisphosphate aldolase, but lack a phosphoketolase. Gluconate of pentoses are not fermented. Group II: Facultatively heterofermentative lactobacilli. Hexoses are almost exclusively fermented to lactic acid by the Embden-Meyerhof-Parnas pathway (EMP). The species possess both a fructose-1.6-bisphosphate aldolase and a phosphoketolase. Consequently, the species can ferment hexoses and pentoses as well as gluconate. In the presence of glucose the enzymes of the phosphogluconate pathway are repressed. Group III: Obligately heterofermentative lactobacilli. Hexoses are fermented by the phosphogluconate pathway yielding lactic acid, ethanol/acetic acid and CO_2 in nearly equimolar amounts. Pentoses are fermented by the same pathway

[a]formation of acetate and formate from lactate or pyruvate, or acetate and CO_2 in the presence of oxidants;

[b]high tolerance to ethanol and acidity;

[c]nitrate reduction, presence of pseudocatalase;

[d]subsp. *Lactis*;

[e]subsp. *Paracasei*; *N.d.* no data given

The Embden-Meyerhof-Parnas pathway is used by lactobacilli (group I and II; Table 1.3) and pediococci, while group III of lactobacilli, leuconostocs and oenococci use the 6-phosphogluconate/phosphoketolase pathway (other designations: pentose phosphate pathway, pentose phosphoketolase pathway, hexose monophosphate pathway). Changes in the end product composition can be influenced by environmental factors. Depending on the growth conditions the end products of homofermenters can be changed largely. In addition to glucose, the hexoses mannose, fructose and galactose may be fermented after isomerisation and/or phosphorylation. Galactose is used via the tagatose pathway by e.g. *Lb. casei*.

Under anaerobic conditions pyruvate can be metabolized by *Lb. casei* to formate and acetate/ethanol (pyruvate formate lyase system) under glucose limitation. End produts are lactate, acetate, formate and ethanol (mixed acid fermentation). Under aerobic conditions *Lb. plantarum* can convert pyruvate to CO_2 and acetyl phosphate with a pyruvate oxidase (Sedewitz et al. 1984).

Flavin-containing enzymes such as NADH:H_2O_2 oxidase and NADH:H_2O oxidase (Condon 1987) can occur in lactic acid bacteria. Oxygen acts as external electron acceptor. Oxygen-dependent glycerol fermentation by *P. pentosaceus* and mannitol fermentation of *Lb. casei* are examples. An oxygen-dependent lactate metabolism has been proposed for *Lb. plantarum* involving NAD^+-dependent and/or NAD^+-independent lactate dehydrogenase, a pyruvate oxidase and an acetate kinase (Murphy et al. 1985).

Lactobacilli interact with oxygen. Some lactic acid bacteria use high intracellular manganese concentration for protection against superoxide (30–35 mM; Archibald 1986). Theobald et al. (2005) found a growth stimulation of *O. oeni* at concentrations of 68 µM or 34 mM manganese in the growth medium. In some strains 34 mM manganese could replace tomato juice. Other compounds are also stimulatory for oenococci (Theobald et al. 2007a, b).

Citrate can lead to diacetyl/actoin formation if the excess of pyruvate is reduced to lactic acid. Oxaloacetate can also function as electron acceptor leading to succinic acid formation when *Lb. plantarum* was grown on mannitol (Chen and McFeeters 1986). *Lb. brevis* and *Lb. buchneri* can use glycerol as electron acceptor in an anaerobic cofermentation with glucose leading to lactate, acetate, CO_2 and 1.3-propandiol (Schütz and Radler 1984a, b). Fructose can be fermented via the 6-phosphocluconate/phosphoketolase pathway and function as electron acceptor to yield mannitol by *Lb. brevis* (Eltz and Vandemark 1960). Malic acid can be used as sole energy source by *Lb. casei* yielding acetate, ethanol and CO_2 or it can be converted to L-lactate and CO_2 (malolactic fermentation) by e.g. *O. oeni* (Radler 1975). The biosynthesis of amino acids in lactic acid bacteria is limited. Some have peptidases and can hydrolyse proteins. Lactic acid bacteria can also perform chemical cell communication (Nakayama and Sonomoto 2002).

1.5 Genetics

The genome size of lactic acid bacteria varies (Morelli et al. 2004). The genome of *Lb. paracasei* consists of 3.4 Mb (Ferrero et al. 1996) and that of *Lb. plantarum* of 3.4 Mb (Chevallier et al. 1994). Restriction maps have been obtained from *O. oeni* (Ze-Ze et al. 2000). The total genome of more than 20 lactic acid bacteria is available, including the wine-related strains *Lc. mesenteroides*, *Lb. plantarum*, *Lb. brevis*, *Lb. paracasei*, *Lb. casei*, *O. oeni* and *P. pentosaceus* (Makarova et al. 2006).

Lactic acid bacteria possess circular as well as linear plasmids associated with carbohydrate fermentation and proteinase activities, bacteriocin production, phage defense mechanisms, and antibiotic resistance mechanisms (Morelli et al. 2004).

Phages have been found with the wine-related species of *Lactobacillus* (*Lb. casei*, *Lb. fermentum*, *Lb. plantarum*,), *Leuconostoc* (*Lc. mesenteroides*) and *Oenococcus* (*O. oeni*) (Josephsen and Neve 2004). They can cause stuck malolactic fermentation (Poblet-Icart et al. 1998).

1.6 Activities in Must and Wine

Lactic acid bacteria are involved in food and feed fermentation and preservation as well as food digestion in the intestinal tracts of humans and animals. Due to its tolerance against ethanol and acidic conditions, LAB can grow in must. Generally they are inhibited at ethanol concentrations above 8 vol%, but *O. oeni* tolerates 14

vol% and *Lb. brevis*, *Lb. fructivorans* and *Lb. hilgardii* can be found even in fortified wines up to an ethanol concentration of 20 vol%. Slime-producing strains of *P. damnosus* grow up to 12 vol% of ethanol. Lactic acid bacteria isolated from wine grow between 15 and 45°C in the laboratory with an optimal growth range between 20 and 37°C. Best growth in must during malolactic fermentation is obtained around 20°C. During the first days of must fermentation the CFU of LAB increases from 10^2 to 10^4–10^5 per ml. After the alcoholic fermentation and during the malic acid fermentation, the cell number can reach a titer of 10^7–10^8 CFU per ml (Ribérau-Gayan et al. 2006a, b). The titer of different lactic acid species during alcoholic fermentation has been determined by Lonvaud-Funel et al. (1991): *O. oeni*, 3.4×10^6 (day 13, alcohol content: 18 vol%); *Lc. mesenteroides*, 9.6×10^4 (day 6, alcohol content: 9 vol%); *P. damnosus*, 3.8×10^4 (day 3, alcohol content: 7 vol%); *Lb. hilgardii*, 8.0×10^4 (day 3, alcohol content: 7 vol%); *Lb. brevis*, 2.0×10^4 (day 3, alcohol content: 7 vol%) and *Lb. plantarum*, 2.0×10^4 (day 3, alcohol content: 7 vol%).

Lactic acid bacteria gain their energy mainly from sugar fermentation. They use both main hexoses of the wine, glucose and fructose, as energy and carbon source. In this respect they are competitors of the ethanol producing yeast *Saccharomyces cerevisiae*. The heterofermentative LAB in wine can also use the pentoses (arabinose, xylose, ribose), which occur in minor concentrations in wine.

Lactic acid bacteria also metabolize the three main acids of must: tartrate, malate and citrate. Citrate is converted to lactate, acetic acid, CO_2 and acetoin. Malate is converted to L-lactate and CO_2 (malolactic fermentation). Especially in northern countries, where must can have high acidity, the biological reduction with starter cultures of *O. oeni* is an important step in vinification. The malolactic enzyme has been found in many lactic acid bacteria occurring in wine (e.g. *Lb. casei*, *Lb. brevis*, *Lb. buchneri*, *Lb. delbruechii*, *Lb. hilgardii*, *Lb. plantarum*, *Lc. mesenteroides, and O. oeni*). *O. oeni* is applied for reduction of the malic acid content because of its high tolerance against ethanol and acidity. Malolactic fermentation and the use of sugars can lead to a more stable wine. Tartrate can be converted to lactate, acetate and CO_2 by the homofermentative lactic acid bacterium *Lb. plantarum* and to acetate and CO_2 or fumaric acid (succinic acid) by the heterofermentative lactic acid bacterium *Lb. brevis* (Radler and Yannissis 1972).

Lactic acid bacteria produce different biogenic amines. *O. oeni*, *P. cerevisiae* and *Lb. hilgardii* (Landete et al. 2005; Mangani et al. 2005) are examples of producers of biogenic amines. The most important is histamine, which is produced by decarboxylation of histidine. The COST Action 917 (2000–2001) of the EU "Biologically active amines in food" suggested prescriptive limits for histamine (e.g. France: $8\,mg\,l^{-1}$, Germany: $2\,mg\,l^{-1}$) in wines. Biogenic amines can cause health problems (Coton et al. 1998) and sensory defects in wine (Lehtonen 1996; Palacios et al. 2004). From arginine, ammonium is liberated by heterofermentative species such as *Lb. higardii* and *O. oeni*, but also by facultatively heterofermentative species like *Lb. plantarum*.

Lactic acid bacteria have an influence on the flavour of wine, because they can produce acetic acid, diacetyl, acetoin, 2,3- butandiol, ethyl lactate, diethyl succinate and acrolein. They cause a decrease in colour up to 30%. In German wines 1.08 g acetic acid per l white wine or 1.20 g acetic acid per l red wine are the upper limits for acetic acid, while e.g. "Beerenauslese" (German quality distinction) can even have higher concentrations. The natural value is 0.3–0.4 g l^{-1} and it becomes sensory-significant at concentrations above 0.6 g l^{-1}. Aerobic acetic acid bacteria, facultatively anaerobic heterotrophic lactic acid bacteria, yeast under difficult fermentation conditions and *Botrytis cinerea* on infected grapes are the potential producers. Fructose is reduced to mannitol or converted to erythrol and acetate. Heterofermentative lactic acid bacteria can produce higher concentrations of acetic acid (>0.6 g L^{-1}), especially in the absence of pantothenic acid (Richter et al. 2001). Lactic acid bacteria can convert sorbic acid, which is used because of its antifungal properties, to 2-ethoxy-3.5-hexadiene (geranium-like odour) (Crowel and Guymon 1975). Glycerol is converted to propandiol-1.3 or allylalcohol and acrolein leading to bitterness (Schütz and Radler 1984a, b). Off-flavour is produced by *O. oeni* from cysteine and methionine. Cysteine is transformed into hydrogen sulfide or 2-sulfanyl ethanol and methionine into dimethyl disulfide, propan-1-ol, and 3-(methasulfanyl) propionic acid. They increase the complexity of the bouquet. The latter has an earthy, red-berry fruit flavour (Ribéreau-Gayon et al. 2006a, b). Lactic acid bacteria may produce a smell reminiscent of mice (mousiness). Species of *Lactobacillus* such as *Lb. brevis*, *Lb. hilgardii* and *Lb. fermentum* produce 2-acetyltetrahydropyridine (perception threshold: 1.6 ng l^{-1}) from ethanol and lysine (Heresztyn 1986). Also 2-acetyl-1-pyrroline and 2-ethyltetrahydropyridine can contribute to this off-flavour (Costello and Henschke 2002). Ethyl carbamate is produced from urea and ethanol by *O. oeni* and *Lb. hilgardii* (Uthurry et al. 2006), which probably is carcinogenic.

Polysaccharide production (Claus 2007) leads to graisse of the must, which causes problems during filtration. *P. damnosus* increases viscosity. It produces a glucose homopolymer. The repeating unit is a β-1.3 linked glucose disaccharide carrying a β-1.2 linked glucose site group [3)-β-D-Glc*p*-(1.3)-[β-D-Glc*p*-(1.2)]-β-D-Glc*p*-(1] (Llaubères et al. 1990; Dueñas et al. 2003). The viscosity, which is influenced by many factors such as the ethanol concentration and temperature, becomes apparent at 10^7 colony forming units.

Lactic acid disease occurs at higher sugar concentrations when lactic acid bacteria grow during ethanolic fermentation at higher pH values and low nitrogen concentrations. Higher amounts of acetic acid can be produced, which hampers the activities of yeast. Most often, LAB do not multiply or disappear during alcoholic fermentation, except oenococci, which resist at low cell levels. It was found that fatty acids (hexanoic, octanoic and decanoic acid) liberated by growing yeast have a negative effect on bacterial growth (Lonvaud-Funel et al. 1988). Oenococci can grow during the stationary/death phase of the yeasts after alcoholic fermentation, when released cell constituents of yeasts stimulate bacterial growth. In this stage oenococci have an influence on yeast lysis by producing glycosidases and proteases.

The degradation of sugars and acids contributes to the microbial stabilisation of wine by removing carbon and energy substrates. Low concentrations of diacetyl increase the aromatic complexity. If the concentration of volatile acids increases 1 g l^{-1} the lactic disease becomes apparent, which can lead to a stuck alcoholic fermentation.

Lactic acid bacteria potentially produce antimicrobial components (Rammelberg and Radler 1990; Blom and Mörtvedt 1991) such as acetic acid, higher concentrations of carbon dioxide, hydrogen peroxide, diacetyl, pyroglutamic acid and bacteriocins, which inhibit the growth of other bacterial and yeast species. Brevicin from *Lb. brevis* inhibits growth of *Oenococus oeni* and *P. damnosus* (Rammelberg and Radler 1990).

The malolactic fermentation and the consumption of nutrients (hexoses and pentoses) as well as the production of bacteriocines (De Vuyst and Vandamme 1994) lead to a stabilization of wine.

1.7 Characteristics of Genera and Species of Wine-Related Lactic Acid Bacteria

1.7.1 Genus Lactobacillus

Lactobacillus is one of the most important genera involved in food microbiology and human nutrition, owing to their role in food and feed production and preservation, as well as their probiotic properties. In October 2008 this genus contained in total 174 validly described species (including subspecies) (DSMZ 2008). *Lactobacillus* species live widespread in fermentable material. Lactobacilli contribute to the flavour of fermented food by the production of diacetyl, H_2S and amines. They play a role in the production as well in the spoilage of food (sauerkraut, silage, dairy and meat as well as fish products) and beverages (beer, wine, juices) (Kandler and Weiss 1986; Hammes et al. 1991).

Lactobacilli are straight gram-positive non-motile or rarely motile rods (e.g. *Lb. mail*), with a form sometimes like coccobacilli. Chains are commonly formed. The tendency towards chain formation varies between species and even strains. It depends on the growth phase and the pH of the medium. The length and curvature of the rods depend on the composition of the medium and the oxygen tension. Peritrichous flagellation occurs only in a few species, which is lost during growth in artificial media. They are aciduric or acidophilic. The maximum for growth pH is about 7.2.

The murein sacculi possess various peptidoglycan types (Lys-D-Asp, m-Dpm-direct, Orn-D-Asp, Lys-Ala, Lys-Ala_2, Lys-Ala-Ser, Lys-Ser Ala_2) of group A. Polysaccharides are often observed. Membrane-bound teichoic acids are present in all species and cell wall-bound teichoic acids in some species (Schleifer and Kandler 1972).

The G + C content of the DNA ranges from 32 to 53 mol%.

Lactobacilli are strict fermenters. They can tolerate oxygen or live anaerobic. They have complex nutritional requirements for carbohydrates, amino acids, peptides, fatty acids, nucleic acid derivatives, vitamins and minerals.

Some species possess a pseudocatalase and some strains can take up porphorinoids and then exhibit catalase, nitrite reductase and cytochrome activities.

They gain energy by homofermentative or heterofermentative carbohydrate fermentation in the absence or presence of oxygen. An energy source is also the conversion of carbamyl phosphate to CO_2 and NH_3 during arginine degradation. They possess flavine-containing oxidases and peroxidases to carry out an oxidation with O_2 as the final electron acceptor. The pathways of sugar fermentation are the Embden-Meyerhof pathway converting 1 mol hexose to 2 mol lactic acid (homolactic fermentation) and the phosphoketolase pathway (heterolactic fermentation) resulting in 1 mol lactic acid, ethanol/acetate and CO_2. Pyruvate produced during hexose fermentaion may be converted to lactate, but also to other products such as diacetyl or acetic acid, ethanol and formate/CO_2. In the presence of oxygen, lactate can be converted to pyruvate and consequently to acetic acid and CO_2 or acetate and formate. The conversion of glycerol to 1,3-propanediol with glucose serving as electron donor was observed in *Lb. brevis* isolated from wine (Schütz and Radler 1984a, b). The homofermentative species possess an FDP aldolase, while the heterofermentative species have a phosphoketolase. The facultative heterofermenters possess an inducible phosphoketolase. Heterofermentative species can also use pentoses as substrate. Some homofermenters use pentores homofermentatively (Rodas et al. 2006)

Sucrose is also used for the formation of dextrans with the help of dextran sucrase. Fructose can serve as electron acceptor and mannitol is formed by heterofermentative species. Monomeric sugars and saccharides are taken up by permeases or the phosphotransferase system. They are split inside the cell by glycosidases. Galactose-6-phosphate from lactose phosphate is fermented via the tagatose-6-phosphate pathway (Kandler 1983). Several organic acids such as citric acid, tartaric acid or malic acid are degraded (Radler 1975). Several amino acids are decarboxylated to biogenic amines.

Depending on the stereospecificity of the lactate dehydrogenase or the presence of an inducible lactate racemase lactate may have the D(−) or L(+) configuration. The lactate dehydrogenases can differ with respect to electrophoretic mobility and kinetic properties. Some enzymes are allosteric with FDP and Mn^{2+} as effectors.

Plasmids linked to drug resistance or lactose metabolism are often found (Smiley and Fryder 1978). Double-stranded DNA phages have been isolated (Sozzi et al. 1981) and lysogeny is widespread (Yokokura et al. 1974). Strains producing bacteriocins (lactocins) have been found among the homo- and heterofermentative species (Tagg et al. 1976). Several serological groups have been designed. From the species in must, *Lb. plantarum* belongs to group D (antigen: ribitol teichoic acid), *Lb. fermentum* to group F and *Lb. brevis* to group E (Archibald and Coapes 1971).

The complete genome of eleven *Lactobacillus*-species has been sequenced; it includes the wine related species *Lb. casei* and *Lb. plantarum* (http://www.ncbi.nlm.nih.gov/genomes/lproks.cgi).

Some characteristics of the species are compiled in Table 1.3. A combination of physiological and biochemical as well as molecular tests are required for the unambiguous identification of *Lactobacillus* species (Pot et al. 1994; Hammes and Vogel 1995). Hundred and fifteen validly published species of the genus *Lactobacillus* can be assigned to nine groups (cf. Table 1.3) (Yang and Woese 1989; Collins et al. 1991; Hammes et al. 1991; Hammes and Vogel 1995; Dellaglio and Felis 2005). Out of about 174 described species/subspecies, sixteen have been found in must and wine (Table 1.3) (Ribéreau-Gayon et al. 2006 a, b; Fugelsang and Edwards 2007).

The type species is *Lb. delbrueckii* DSM 20074^T.

Lb. brevis
Morphology: Rods. 0.7–1.0 µm × 2.0–4.0 µm. Single or chains.
Isolation: Milk, cheese, sauerkraut, sourdough, silage, cow manure, mouth, intestinal tract of humans and rats, grape must/wine.
Type strain: DSM 20054.

Lb. buchneri
Morphology: Rods. 0.7–1.0 µm × 2.0–4.0 µm. Single or short chains.
Characteristics: As described for *Lb. brevis* except the additional fermentation of melezitose and the distinct electrophoretic behaviour of L-LDH and D-LDH.
Isolation: Milk, cheese, plant material and human mouth, grape must/wine.
Type strain: DSM 20057.

Lb. casei
Morphology: Rods. 0.7–1.1 µm × 2.0–4.0 µm.
Isolation: Milk, cheese, dairy products, sour dough, cow dung, silage, human intestinal tract, mouth and vagina, sewage, grape must/wine.
Type strain: DSM 20011.

Lb. cellobiosus
→ *Lb. fermentum*.

Lb. curvatus
Morphology: Bean-shaped rods. 0.7–0.9 µm × 1.0–2,0 µm. Pairs, short chains or close rings. Sometimes motile.
Characteristics: LDH is activated by FDP and Mn^{2+}. Lactic acid racemase.
Isolation: Cow dung, milk, silage, sauerkraut, dough, meat products, grape must/wine.
Type strain: DSM 20019 (subsp. *curvatus*).

Lb. delbrueckii
Morphology: Rods. 0.5–0.8 µm × 2.0–9.0 µm. Single or in short chains.
Isolation: Milk, cheese, yeast, grain mash, grape must/wine.
Type strain: DSM 20072 (subsp. *lactis*).