Chapter 1
Lactic Acid Bacteria

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1.1 Introduction

In 1873, 10 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 5000 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 is 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

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

Family	Genus	Species from must and wine
I. "Aerococcaceae"	I. *Abiotrophia* II. *Aerococcus* III. *Dolosicoccus* IV. *Eremococcus* V. *Facklamia* VI. *Globicatella* VII. *Ignavigranum*	
II. "Carnobacteriaceae"	I. *Alkalibacterium* II. *Allofustis* III. *Alloiococcus* IV. *Atopobacter* V. *Atopococcus* VI. *Atopostipes* VII. *Carnobacterium* VIII. *Desemzia* IX. *Dolosigranulum* X. *Granulicatella* XI. *Isobaculum* XII. *Marinilactibacillus* XIII. *Trichococcus*	
III. "Enterococcaceae"	I. *Enterococcus* II. *Melissococcus* III. *Tetragenococcus* IV. *Vagococcus*	*E. faecium*
IV. Lactobacillaceae	I. *Lactobacillus*[b]	*Lb. brevis*, *Lb. buchneri*, *Lb. casei*, *Lb. curvatus*, *Lb. delbrueckii*, *Lb. diolivorans*, *Lb. fermentum*, *Lb. florum*, *Lb. fructivorans*, *Lb. hilgardii*, *Lb. jensenii*, *Lb. kunkeei*, *Lb. mali*, *Lb. nagelii*, *Lb. oeni*, *Lb. paracasei*, *Lb. plantarum*, *Lb. vini*
	II. *Paralactobacillus*	
	III. *Pediococcus*	*P. damnosus, P. inopinatus, P. parvulus*, *P. pentosaceus*
V. "Leuconostocaceae"	I. *Leuconostoc* II. *Oenococcus* III. *Weissella*	*Lc. mesenteroides* *O. oeni* *W. paramesenteroides*
VI. Streptococcaceae	I. *Lactococcus*[b] II. *Lactovum* III. *Streptococcus*	

[a]Garrity (2005), Vos et al. (2009), Whitman (2016), DSMZ (2016b)

[b]Species of *Enterococcus* and *Lactococcus* (*Lcc. lactis*) have been found on grapes (Bae et al. 2006; Nisiotou et al. 2015). *Enterococcus faecium* was identified in fermenting must (Pérez-Martín et al. 2014). Species of these two genera are not further considered here. In addition, *Lb. graminis* (Nisiotou et al. 2015) and *W. uvarum* (Nisiotou et al. 2014) have been isolated from grapes

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*

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 33 described genera, only 26 species belonging to six genera have been isolated from must and wine (Table 1.1).

The homofermentative species produce lactic acid (<85%) as the sole end product, while the heterofermentative species produce lactic acid, CO_2 and ethanol/acetate from glucose. 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, 1.3, 1.4 and 1.5).

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; Lahtinen et al. 2012) and their activities on grape or in must and wine (Fleet 1993; Dittrich and Großmann 2005, 2011; Ribéreau-Gayon et al. 2006a, b; Fugelsang and Edwards 2007) has been compiled in several books. Here we concentrate mainly on lactic acid bacteria found in fermenting must and wine.

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,

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*[b]	*Lb. diolivorans*	*Lb. fermentum*	*Lb. fructivorans*[c]
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*[c]	*Lb. jensenii*	*Lb. kunkeei*	*Lb. mali*	*Lb. nagelii*	*Lb. paracasei*[d]	*Lb. plantarum*[e]	*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
Glycerol teichoic acid	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.	–	–	+	–

(continued)

Table 1.3 (continued)

Characteristics	*Lb. hilgardii*[c]	*Lb. jensenii*	*Lb. kunkeei*	*Lb. mali*	*Lb. nagelii*	*Lb. paracasei*[d]	*Lb. plantarum*[e]	*Lb. vini*
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 (2005) and Felis and Dellaglio (2007) (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 the Embden–Meyerhof–Parnas pathway (EMP). The organisms possess a fructose-1.6-bisphosphate aldolase, but lack a phosphoketolase. Gluconate or 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]Subsp. *Lactis*

[c]High tolerance to ethanol and acidity

[d]Subsp. Paracasei

[e]Nitrate reduction, presence of pseudocatalase

n.d. No data given

The characteristics of the newly described species *Lb. florum* (Endo et al. 2010) and *Lb. oeni* (Mañes-Lázaro et al. 2009) are summarized in Sect. 1.7.1

Table 1.4 Differential characteristics of wine-related species of the genera *Leuconostoc*, *Oenococcus* and *Weissella*

Characteristics	*Lc. mesenteroides*	*O. oeni*	*W. paramesenteroides*
Acid from sucrose	+	–	+
Dextran formation	+	–	–
Growth below pH 3.5	–	+	n.d.
Growth in 10% ethanol	–	+	n.d.
NAD^+-dependent Glc-6-P-DH	+	–	n.d.
Murein type	Lys-Ser-Ala_2	Lys-Ser_2, Lys-Ala-Ser	Lys-Ser-Ala_2, Lys-Ala_2

n.d. Data not given

Table 1.5 Differential characteristics of wine-related species of the genus *Pediococcus*

Characteristics	*P. damnosus*	*P. inopinatus*	*P. parvulus*	*P. pentosaceus*
Mol% G+C	37–42	39–40	40.5–41.6	35–39
Growth at/in				
35 °C	–	+	+	+
6% NaCl	–	+	+	+
pH 8.0	–	–	–	+
Arginine hydrolysis	–	–	–	+
Acid from				
Arabinose	–	–	–	+

Pedicocci can be identified by multiplex PCR (Pfannebecker and Fröhlich 2008)

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). *O. oeni* strains induced strain-specific cytokine patterns measureable immunomodulatory potential (Foligné et al. 2010).

Lactic acid bacteria can also be found on grapes, in grape must and wine, as well as 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.*) (Tables 1.1 and 1.2) and the

acetic acid genera *Acetobacter*, *Gluconobacter* and *Gluconoacetobacter* 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*, *Streptococcus* and *Bifidobacterium* have not been isolated from must and wine, but sometimes also species of the genus *Enterococcus* (*E. faecium*) could be detected in wine (Pérez-Martín et al. 2014).

Detailed investigations of the grape associated bacteria have been undertaken (Jackson 2008). Species of the lactic acid genera *Lactobacillus* (*Lb. casei*, *Lb. hilgardii*, *Lb. kefiri*, *Lb. kunkeei*, *Lb. lindneri*, *Lb. mali*, *Lb. plantarum*), *Weissella paramesenteroides*, *Enterococcus* (*E. avium*, *E. durans*, *E. faecium*, *E. hermanniensis*), *Lactococcus lactis* and infrequently species of the acetic acid genera *Asaia* and *Gluconobacter* as well as grampositive genera *Bacillus* and *Staphylococcus* have been identified in enrichment cultures from undamaged or damaged grapes of the varieties (Cabernet Sauvignon, Chardonnay, Pinot Noir, Sauvignon Blanc, Semillion, Shiraz, Tyrian) in Australia (Bae et al. 2006). Vineyard- and winery-associated lactic acid bacteria (LAB) from the Greek wine growing regions Peza and Nemea revealed that *Pediococcus pentosaceus* and *Lb. graminis* dominated the grape microbiota and *Lb. plantarum* the fermenting must (Nisiotou et al. 2015). Species of the genera *Enterococcus* and *Lactococcus* are not further considered here.

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, menaquinone type, fatty acid composition, electrophoretic mobility of the lactate dehydrogenases, DNA base composition, PCR-based fingerprinting techniques (SAPD-PCR; Pfannebecker and Fröhlich 2008; Sebastian et al. 2011; Petri et al. 2013), restriction analysis (Ze-Ze et al. 2000), restriction fragment length polymorphism (PCR-RFLP) analysis of 16S ribosomal RNA (rRNA) genes (Ilabaca et al. 2014), 16S-ARDRA (Rodas et al. 2003), DNA–DNA homology, soluble protein pattern, 16S rDNA and gene sequencing (e.g. *recA*) (Axelsson 2004), multilocus sequence typing (MLST) and pulsed field gel electrophoresis analysis (PFGE) (González-Arenzana et al. 2014), quantitative PCR (Cho et al. 2011), marker-targeted quantitative PCR (Solieri and Giudici 2010), amplification of 16S rRNA

gene restriction with the endonuclease FseI (Marques et al. 2010), real-time PCR (Kántor et al. 2016), fluorescence in situ hybridization (FISH; Hirschhäuser et al. 2005), mass spectrometry (Napoli et al. 2014; Petri et al. 2015), multiplex PCR (Pfannebecker and Fröhlich 2008; Petri et al. 2013) and complete genome comparison (GGDC - The Genome-to-Genome Distance Calculator; DSMZ 2016d). qPCR after propidium monoazide treatment of samples is a rapid tool to enumerate *O. oeni* viable cells with intact membranes in must and wine (Vendrame et al. 2013).

The genera and species of lactic acid bacteria occurring in must and wine can be differentiated by phenotypic features (Tables 1.2, 1.3, 1.4 and 1.5). The species can be identified by the API 50 CHL identification system (Bio-Mérieux) or the Biolog Microbial Identification System (Biolog, Inc.) (Testa et al. 2014).

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. 1.1; 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

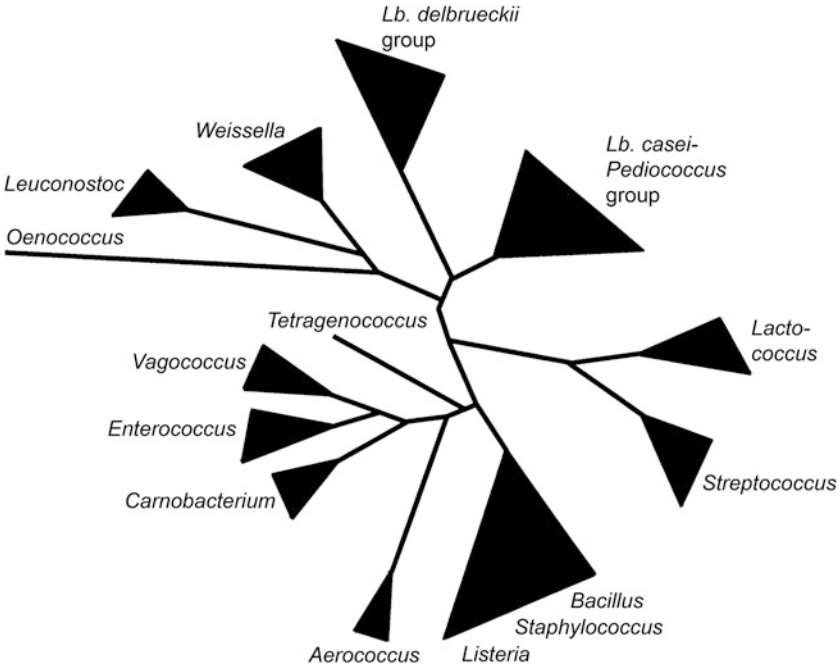

Fig. 1.1 Schematic unrooted phylogenetic tree of lactic acid bacteria and related genera (Axelsson 2004; with permission of the author and the publisher)

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 three species *O. oeni* and *O. kitahareae* (Endo and Okada 2006) as well as *O. alcoholitolerans* (Badotti et al. 2015). *O. kitahareae* 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. *O. alcoholitolerans* thrived in an ethanol production plant in Brazil.

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

Today, the lactic acid bacteria are members of the domain Bacteria, where they are assigned to the phylum Firmicutes, the class Bacilli and the order Lactobacillases (Table 1.1) (Garrity 2005); Vos et al. 2009; Whitman 2016).

1.4 Physiology

Carbohydrates are used as carbon and energy source by a homofermentative or heterofermentative pathway. Fructophilic species have been described (Endo and Okada 2008; Mtshali et al. 2012). Sugars or oligosaccharides 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-phosphogluconate/phosphoketolase pathway leading to lactic acid and acetic acid/ethanol. Some lactobacilli 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).

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). The defense system against in vitro oxidative stress includes the 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging ability, reactive oxygen species (ROS) scavenging ability, iron ion chelation (FE), glutathione system, ferric reducing ability of plasma (FRAP), reduction activity (RA), inhibition of ascorbic oxidation (TAA), and linoleic acid oxidation (TLA) abilities (Su et al. 2015).

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).

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).

Citrate can lead to diacetyl/acetoin 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).

Adaptation of lactobacilli to harsh environmental conditions concern: synthesis of heat-shock proteins, key enzymes of glycolytic pathways, the glutamate decarboxylase system, homoeostasis of intracellular pH, alkalization of the external environment, DNA and protein damage repair, changes in cell membrane composition, changes in cytosolic and surface-located proteins, the fatty acid contents of the cytoplasmic membrane, cell wall biosynthesis, transport of peptides, coenzyme levels and membrane H+-ATPase (Hussain et al. 2013).

1.5 Genetics

The genome size of lactic acid bacteria varies (Morelli et al. 2004). The total genome of about 211 species/strains of the genera *Lactobacillus* (genome size: 1.27765–4.87232 Mb), *Leuconostoc* (genome size: 1.63897–2.29809 Mb), *Oenococcus* (genome size: 1.15038–1.84224 Mb), *Pediococcus* (genome size: 1.76496–2.50947 Mb) and *Weissella* (genome size: 1.33444–2.57773 Mb) is available, including all wine-relevant species (Table 1.1; Makarova et al. 2006; https://www.ncbi.nlm.nih.gov/genome, February 2017). For example, the genome of *Lb. paracasei* ATCC 334 consists of 2.17 Mb (Ferrero et al. 1996) and that of *Lb. plantarum* CCM 1904 of 3.4 Mb (Chevallier et al. 1994). Genome sequences of *O. oeni* strains have been determined (Jara and Romero 2015).

Lactic acid bacteria (LAB) have about 2000 genes in average. They have lost ca. 1000 genes during separation from the ancestral Bacilli during evolution. The lost genes coded for sporulation, cofactors, heme cytochromes and catalase. LAB have also acquired about 86 new genes by gene duplication and horizontal gene transfer regarding e.g. murein and B12 biosynthesis, novel functions of genes coding usually for antibiotic resistance, phage defense mechanisms and IS elements (Morelli et al. 2012).

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, 2012).

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 their 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 ml^{-1}. 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. Indigenous *P. damnosus* strains were found to perform malolactic fermentation into Albariño and Caiño wines (Spain) without negative effects on the wine (Juega et al. 2014). Malolactic fermentation and the use of sugars can lead to a more stable wine. *Lb. plantarum* could be an alternative species to *O. oeni* for performing malolactic fermentation (Bravo-Ferrada et al. 2013). 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; Kaschak et al. 2009; Sebastian et al. 2011; Christ et al. 2012) 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*. The highest citrulline production in Malbec wine could be correlated with its lower concentrations of glucose, fructose, citric and phenolic acid than the other wines. Therefore, a wine with lower concentration of these sugars and acids could be dangerous due to the formation of ethyl carbamate precursors. The degradation if arginine proceeds via citrulline that forms with ethanol the carcinogen ethyl carbamate. Phenolic compounds could decrease the arginine consumption (protocatechuic acid, gallic acid) or increase (quercetin, rutin, catechin, caffeic acid, vanillic acids). Arginine deiminase activity was stimulated by rutin, quercetin, caffeic acid and vanillic, while gallic acid and protocatechuic acids inhibited this enzyme activity (Alberto et al. 2012; Araque et al. 2016). Nuclear magnetic resonance (NMR) spectroscopy is a tool to follow the transformation of histidine into histaminol and into histamine during alcoholic and malolactic fermentations and consequently to select suitable strains for malolactic fermentation (López-Rituerto et al. 2013). On the other hand biogenic amines such as histamine, tyramine, and putrescine can be degraded by lactic acid bacteria (e.g. *Lb. plantarum*, *P. acidilactici*) (Callejón et al. 2014), which is also true for some yeasts (Bäumlisberger et al. 2015). Strains of *Lb. plantarum* were selected because of their ability to degrade putrescine and tyramine (Capozzi et al. 2012). Although at different extent, 25% of the LAB especially *Lactobacillus* and *Pediococcus* strains were able to degrade histamine, 18% tyramine and 18% putrescine, whereas none of the commercial malolactic starter cultures or type strains were able to degrade any of the tested amines. The application of some lactic acid bacteria could be a promising strategy to reduce biogenic amines in wine (García-Ruiz et al. 2011a).

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. The ability of wine lactobacilli to accumulate 3-hydroxypropionaldehyde (3-HPA), a precursor of acrolein, from glycerol in the fermentation media was demonstrated (Bauer et al. 2010). Lactic acid bacteria can also 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; Arena et al. 2013), which probably is carcinogenic. Lactic acid bacteria possess esterases for the synthesis and hydrolysis of esters (Sumby et al. 2013). *Lb. plantarum* posseses arylesterase which showed high hydrolytic activity on phenyl acetate and lower activity on other relevant wine aroma compounds (Esteban-Torres et al. 2014). Commercial strains of *Oenococcus oeni* and *Lb. plantar*um synthesize flavour active fatty acid ethyl esters with the aid of an acyl coenzyme A: alcohol acyltransferase (AcoAAAT) activity and a reverse esterase activity leading to an increased ethyl ester content of wine (Costello et al. 2013). The polyphenol flavan-3-ol was metabolized by *Lb. plantarum* to phenylpropionic acids (Barroso et al. 2014). In general, flavonols and stilbenes showed the greatest inhibitory effects among wine polyphenols on *O. oeni*, *Lb. hilgardii* and *P. pentosaceus* (García-Ruiz et al. 2011b). The proteome of *Oenococcus oeni* was studied to get hints about metabolic activities that can modify the taste and aromatic properties of wine (Mohedano et al. 2014). *Lb. plantarum* converted p-coumaric acid to volatile phenolic compound 4-vinylphenol under wine related conditions (Fras et al. 2014), reactions described earlier to be performed by intestinal bacteria of termites Kuhnigk et al. 1994). Hydroxycinnamic acids stimulated the production of the volatile phenolic compound 4-vinylphenol from p-coumaric acid by the LAB test strains *Lb. plantarum*, *Lb. collinoides* and *P. pentosaceus* (Silva et al. 2011). Isolates belonging to the genera *Oenococcus*, *Lactobacillus*, *Pediococcus* and *Enterococcus* exhibited intracellular esterase activities using p-nitrophenyl octanoate as test compound. The esterase activity was decreased by increasing ethanol concentrations (Pérez-Martín et al. 2013).

Polysaccharide production (Claus 2007) leads to graisse of the must, which causes problems during filtration. *O. oeni* synthesizes homo- and heteropolysaccharides which are important for the adaptation to the wine environment, but also may influence the wine structure (Dimopoulou et al. 2012). *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. A lytic enzyme for the hydrolysis of the slime produced by *P. parvulus* has been described (Blättel et al. 2011). β-D-Glucosidase activity occurred intracellularly in lactic acid bacteria (Mesas et al. 2012; Pérez-Martín et al. 2012). The application with lysozyme and β-glucanase leads to an improved treatment against glycan producing strains strains (Coulon et al. 2012). Of course, some phenolic compounds are inhibitory for lysozyme (Guzzo et al. 2011). When the S-layer was removed, the corresponding *Lb. hilgardii* B706 cells became more sensitive to bacteriolytic enzymes and some wine-related stress conditions (Dohm et al. 2011).

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. The production of bacteriocins by wine lactobacilli and *L. mesenteroides* is important for the production of wine aroma and combating other spoilage lactobacilli or controlling the malolactic fermentation (Du Toit et al. 2011; Dündar et al. 2016). Brevicin from *Lb. brevis* inhibits growth of *O. 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. Compared to *O. oeni* *Lb. plantarum* possesses more genes encoding for glycosidases, proteases, esterases, phenolic acid decarboxylases and citrate lyases and bacteriocins (plantaricins).

Analysis with DNA microarrays and proteomic techniques revealed that genes associated with the amino acid, the malate and the citrate metabolism, the synthesis

of certain cell wall proteins were up, but genes related to carbohydrate metabolism were down regulated under wine making conditions. In addition, the thioredoxin and glutathione systems played an adaptive function for life (Margalef-Català et al. 2016).

During incubation with proteins and polypeptides obtained from Cabernet Sauvignon and Syrah wines *O. oeni* excreted a proteolytic activity. The produced peptides enhanced the beneficial biological activities in respect to antioxidant and antihypertensive status of the wine (Apud et al. 2013a, b). *O. oeni* could give additional value to wine because of the bioactive peptides from yeast autolysates with multifunctional beneficial activity released as consequence of its proteolytic activity (Aredes Fernández et al. 2011).

The viability of the cells of *O. oeni* is increased when microcolonies are formed. *O. oeni* forms microcolonies on stainless steel and oak chip surfaces with extracellular substances (Bastard et al. 2016). Cell in biofilms possessed increased tolerance to wine stress, and performed effective malolactic activities. Biofilm of *O. oeni* can modulate the wood-wine transfer of volatile aromatic compounds and influence the aging process by decreasing furfural, guaiacol, and eugenol. Most likely, the biofilms consists of polysaccharides, because *O. oeni* produces cell-linked exopolysaccharides (EPS) consisting of glucose, galactose and rhamnose as well as soluble β-glucan and soluble dextran or levan polymers (Dimopoulou et al. 2016). In addition, heat shock proteins contribute to stress reduction under wine conditions. Beside polysaccharide formation heat shock proteins play a role in acid tolerance. Darsonval et al. (2015) applied the antisense RNA approach to revealed the function of the small heat stress protein (HSP) Lo18 of *O. oeni*. They found that Lo18 is involved in heat and acid tolerance, which was explained by its membrane-protective role. The heat shock protein Hsp20 is over-expressed (Olguín et al. 2015; Costantini et al. 2015). Nevertheless, high ethanol concentrations in wine have an effect on metabolite transport as well as cell wall and membrane biogenesis.

The development of certain bacterial and yeast starter cultures for wines with special features is a continuous challenge (du Toit et al. 2011; Sumby et al. 2014). Multicolor capillary electrophoresis was performed to derive genotypic and phenotypic characters from fragment length analysis (FLA) profiles (Claisse and Lonvaud-Funel 2014). To improve strain selection a typing scheme for *O. oeni* using multiple-locus variable number of tandem repeat analysis was developed (Claisse and Lonvaud-Funel 2012). In this context it is desirable to find links between *O. oeni* metabolism, genomic diversity and wine sensory attributes (Bartowsky and Borneman 2011). The genomic diversity is well known among *O. oeni* strains, which possess variations in the starter-culture efficiency.

Some undesirable lactic acid bacteria from wine samples have other positive features. A *P. parvulus* strain that was isolated from Douro wines was able to degrade the prominent mycotoxin Ochratoxin A (OTA) (Abrunhosa et al. 2014) and *P. pentosaceus* exhibited a potential as probiotic (García-Ruiz et al. 2014). Also some unwanted compounds such as copper can be adsorbed of by wine-relevant lactobacilli. About 0.5–1.0 μg copper per ml could be removed from wine samples, which is sufficient enough to lower critical copper concentrations. The highest

binding capacity of the tested lactic acid bacteria was found with *Lb. buchneri* DSM 20057 with a maximum of 46.17 µg copper bound per mg cell in deionized water. (Schut et al. 2011).

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 genus 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 2016 this genus contained in total 189 validly described species (DSMZ 2016a). In addition, several species consist of well characterized subspecies. *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. mali*), 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 (DSMZ 2016c). 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 fermentation 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 pentoses homofermentatively (Rodas et al. 2006). Strains of *Lactobacillus kunkeei* turned out to be fructophilic lactic acid bacteria (Endo et al. 2012).

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 173 *Lactobacillus*-species/strains has been sequenced; it includes all the wine related species of the genus *Lactobacillus* (http://www.ncbi.nlm.nih.gov/genome, February 2017).

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). The validly published species of the genus *Lactobacillus* have been 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 189 validly described species, eighteen species have been found in must and wine (Table 1.3) (Ribéreau-Gayon et al. 2006a, 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*).

Lb. diolivorans
Morphology: Rods. 1.0 μm × 2.0–10.0 μm. Single, pairs and short chains.
Isolation: Maize silage, grape must/wine.
Type strain: DSM 14421.

Lb. fermentum
Morphology: Rods. Diameter 0.5–0.9 μm, length variable. Single or pairs.
Isolation: Yeast, milk products, sourdough, fermenting plant material, manure, sewage, mouth and faeces of man, grape must/wine.
Type strain: DSM 20052.

Lb. florum
Morphology: Rods. 0.8 μm × 1.5–7 μm. Single, pairs, chain.
Characteristics: Catalase negative, except in the presence of sheep blood. Heterofermentative. Production of D,L-lactic acid, ethanol and acetic acid from D-glucose. Nitrate not reduced. Acid production only from D-glucose and D-fructose out of 49 tested sugars. Fructophilic. No acid production from L-arabinose, D-arabitol, *N*-acetylglucosamine, maltose, ribose, D-arabinose, L-arabitol, adonitol, amygdalin, arbutin, cellobiose, dulcitol, aesculin, erythritol, D-fucose, L-fucose, β-gentiobiose, 2- and 5-ketogluconate, methyl α-D-glucoside, glycerol, glycogen, inositol, inulin, D-lyxose, D-mannose, methyl α-D-mannoside, melezitose, raffinose, rhamnose, sucrose, salicin, starch, sorbitol, L-sorbose, D-tagatose, trehalose, turanose, xylitol, L-xylose, methyl β-xyloside, D-galactose, lactose, mannitol, melibiose or D-xylose. No dextran production from sucrose. Growth at 300 g D-fructose per l, between pH 4.0–8.0, in the presence of 5% (w/v) NaCl and at 15 °C, but not at 45 °C. Pyruvate stimulatory. Murein lacks meso-diaminopimelic acid. DNA G+C content: 42 mol%.
Isolation: South African peony and bietou flowers, grape, wine
Type strain: DSM 22689

Lb. fructivorans
Morphology: Rods. 0.5–0.8 μm × 1.5–4.0 μm (occasionally 20 μm). Single, pairs, chains or long curved filaments.
Isolation: Spoiled mayonnaise, salad dressing, vinegar preserves, spoiled sake, dessert wine and aperitifs.
Type strain: DSM 20203.

Lb. heterohiochii
→ *Lb. fructivorans*.

Lb. hilgardii
Morphology: Rods. 0.5–0.8 μm × 2.0–4.0 μm. Single, short chains or long filaments.
Isolation: Wine samples.
Type strain: DSM 20176.

Lb. jensenii
Morphology: Rods. 0.6–0.8 μm × 2.0–4.0 μm. Single or short chains.
Isolation: Human vaginal discharge and blood clot, grape must/wine.
Type strain: DSM 20557.

Lb. kunkeei
Morphology: Rod. 0.5 μm × 1.0–1.5 μm.
Characteristics: Week catalase activity.
Isolation: Commercial grape wine undergoing a sluggish/stuck alcoholic fermentation.
Type strain: DSM 12361.

Lb. leichmannii
→ *Lb. delbrueckii* subsp. *lactis*

Lb. mali
Morphology: Slender rods. 0.6 μm × 1.8–4.2 μm, Single, in pairs, palisades and irregular clumps.
Characteristics: Motile by a few peritrichous flagella. Pseudocatalase activity in MRS medium containing 0.1% glucose. Menaquinones with predominantly eight or nine isoprene residues.
Isolation: Apple juice, cider and wine must.
Type strain: DSM 20444.

Lb. nagelli
Morphology: Rods. 0.5 μm × 1.0–1.5 μm.
Characteristics: Nitrate reduction.
Isolation: Partially fermented wine with sluggish alcoholic fermentation.
Type strain: DSM 13675.

Lb. oeni
Morphology: Rods, 0.63–0.92 μm × 1.38–3.41 μm, single, pairs, chains
Characteristics: motile, catalase negative, growth between 15 and 45 °C and pH 4.5–8.0, no growth at 5 °C and pH 3.3. Heterofermentative. Transformation of L-malic acid into L-lactic acid. Gluconate or ribose not fermented. L-Lactate formed from hexoses. Ammonia is not produced from arginine. Fructose not reduced to mannitol. Exopolysaccharide production from sucrose. Acid produced from N-acetylglucosamine, fructose, glucose, mannose, mannitol, sorbitol, L-sorbose, methyl a-D-glucoside and trehalose. No acid production from adonitol, amygdalin, D- or L-arabinose, D- or L-arabitol, arbutin, cellobiose, dulcitol, erythritol, D- or L-fucose, galactose, gluconate, 2-or 5-ketogluconate, glycogen, inositol, inulin, D-lyxose, lactose, maltose, melezitose, melibiose, raffinose, rhamnose, ribose, starch, sucrose, D-tagatose, turanose, xylitol, D- or L-xylose, methyl a-D-mannoside or methyl bxyloside. Aesculin not hydrolysed. Variable usage of glycerol, salicin and gentiobiose. Murein contains D-meso-diaminopimelic acid. DNA G+C content 37.17 mol%.
Isolation: Bobal grape wine
Type strain: DSM 19972

Lb. paracasei
Morphology: Rods. 0.8–1.0 μm × 2.0–4.0 μm. Single or chains.
Isolation: Dairy products, silage, humans, clinical sources, grape must/wine.
Type strain: DSM 5622 (subsp. *paracasei*).

Lb. plantarum
Morphology: Rods. 0.9–1.2 μm × 3.0–8.0 μm. Single, pairs or short chains.
Characteristics: Nitrate can be reduced under glucose limitation and a pH above 6.0. A pseudocatalase may be produced especially under glucose limitation. A ribitol or glycerol teichoic acid can be present in the cell walls.

Isolation: Dairy products, silage, sauerkraut, pickled vegetables, sourdough, cow dung, human mouth, intestinal tract and stool, sewage and grape must. Type strain: DSM 20174.

Lb. trichodes
→ *Lb. fructivorans*.

Lb. vermiforme
→ *Lb. hilgardii*.

Lb. vini
Morphology: Rods. 0.49–0.82 μm × 1.36–2.8 μm. Single, in pairs or in short chains. Motile.
Characteristics: Uses ribore and arabinose homofermentatively. Catalase-negative. Exopolysaccharide is not produced from sucrose.
Isolation: Fermenting grape must.
Type strain: DSM 20605.

Lb. yamanashiensis
→ *Lb. mali*

1.7.2 Genus Leuconostoc

Leuconostocs thrive on plants and sometimes in milk, milk products, meat, sugar cane and other fermented food products. One species, *Lc. mesenteroides,* has been isolated from must. It is nonhemolytic and nonpathogenic to plants and animals (Garvie 1986a). *Leuconostocs* are heterofermentative cocci producing only D-lactic acid from glucose and are unable to produce ammonia from arginine (Björkroth and Holzapfel 2006).

Leuconostocs form spherical or lenticular cells, pairs or chains. The peptidoglycan belongs to type A. The interpeptide bridge of the peptidoglycan consists of Lys-Ser-Ala_2 or Lys-Ala_2.

Sugars are fermented by the 6-P-gluconate/phosphoketolase pathway with D-lactic acid, ethanol/acetate and CO_2 as end products. NAD^+ or $NADP^+$ will serve as coenzyme of the glucose-6-phosphate dehydrogenase. During malolactic fermentation malate is degraded to L-lactate and CO_2. Cells are nonproteolytic. Nitrate is not reduced.

Cells grow in a glucose medium as elongated cocci. Cells are found singly or in pairs, and form short to medium length chains. On solid media, cells form short rods.

Leuconostocs share many features with the heterofermentative lactobacilli (Dellaglio et al. 1995).

Dextrans, which are of industrial importance, are produced by leuconoctocs, especially *Lc. mesenteroides,* from sucrose as substrate.

Leuconostoc species were divided by Garvie (1960) into six different groups according to the fermentation of 19 carbohydrates. Electrophoretic mobilities of enzymes e.g. LDHs, cell protein pattern, cellular fatty acids, DNA base composition and DNA homology are applied for differentiation of the species (Dellaglio et al. 1995). Citrate metabolisms of *Lc. mesenteroides* subsp. *mesenteroides* might be plasmid linked (Cavin et al. 1988). No other phenotypic features were found to be coded on plasmids, while plasmids of *Lactobacillus* and *Pediococcus* code for sugar utilisation, proteinase, nisin, bacteriocins production, drug resistance, slime formation, arginine hydrolysis and bacteriophage resistance (Dellaglio et al. 1995).

Leuconostocs play a role in the organoleptic quality and texture of food such as milk, butter, cheese, meat and wine. *Leuconostocs* can also spoil food, but they often contribute to the flavour of dairy products due to the production of diacetyl from citrate. These strains are used as starter cultures, for e.g., buttermilk and cheese production. They produce gas from glucose, which can change the texture of fermented food. Due to their slow growth and acidification properties, they represent a minor percentage of the LAB in food. They can become predominant when antibiotic agents are present. They can influence the organoleptic behavior of wine.

Lc. mesenteroides subsp. *mesenteroides* (Wibowo et al. 1985), *Lc. mesenteroides* subsp. *dextranicus* (Björkroth and Holzapfel 2006) and *Lc. mesenteroides* subsp. *cremoris* (Yurdugul and Bozoglu 2002) have been isolated from grape must during alcoholic fermentation.

The G+C content of the DNA ranges between 37 and 41 mol%.

The genus *Leuconostoc* contains in total: 13 species (July 2016; DSMZ 2016a). Some species contain well characterized subspecies. Only three subspecies of *Lc. mesenteroides* play a role in must and wine. Some characteristics are compiled in Table 1.4.

The type species is *Lc. mesenteroides* DSM 20343^T.

Lc. mesenteroides* subsp. *cremoris
Morphology: Like *Lc. mesenteroides* subsp. *mesenteroides*, occur often in long chains.
Characteristics: No dextran formation from sucrose.
Isolation: Milk, fermented milk products, grape must/wine.
Type strain: DSM 20346

Lc. mesenteroides* subsp. *dextranicus
Morphology: Like *Lc. mesenteroides* subsp. *mesenteroides*.
Characteristics: Dextran formation to a lesser extent than *Lc. mesenteroides* subsp. *mesenteroides*.
Isolation: Plant material, meat, milk, dairy products, grape must/wine.
Type strain: DSM 203484

Lc. mesenteroides* subsp. *mesenteroides
Morphology: Coccoid cells in milk, elongated cocci in glucose containing culture media. Single, pairs, short to medium chains. Often rod-shaped on solid media.

Characteristics: Production of excess of exopolysaccharides (dextran) from sucrose. Phages have been described (Sozzi et al. 1978).
Isolation: Silage, fermenting olives, sugar milling plants, meat, milk, dairy products, grape must/wine.
Type strain: DSM 20343

1.7.3 Genus Oenococcus

Oenococci have been isolated from must and wine (Garvie 1986a). They form spherical or lenticular cells, pairs or chains. Murein belongs to type A (DSMZ 2016c). The interpeptide bridge contains Lys-Ala-Ser or Lys-Ser-Ser. Only NAD^+ will serve as coenzyme of the glucose-6-phosphate dehydrogenase (Björkroth and Holzapfel 2006). Petri et al. (2015) applied MALDI-TOF-MS and nested SAPD-PCR for the discrimination of *Oenococcus oeni* isolates at the strain level.

Oenococci have been separated from the genus *Leuconostoc* by 16S rDNA sequence analysis (Fig. 1.1; Dicks et al. 1995; Schleifer and Ludwig 1995a, b). Only three species *O. oeni* (Dicks et al. 1995), *O. kitahareae* (Endo and Okada 2006) and *O. alcoholitolerans* (Badotti et al. 2015) have been described (DSMZ 2016a), and can easily be distinguished. *O. kitaharae* (type strain: DSM 17330^T) has been isolated from a composting distilled shochu residue. L-Malate is not decarboxylated to L-lactate and CO_2 in the presence of fermentable sugars. Cells do not grow below pH 4.5 and in 10% ethanol. Growth is not stimulated by tomato juice. The DNA G+C content ranges from 41 to 43 mol%. *O. kitaharae* possess several functions in cellular defence (bacteriocins, antimicrobials, restriction-modification systems), which are lacking in *Oenococcus oeni* living in must with fewer competitive microbes (Borneman et al. 2012). *O. alcoholitolerans* was isolated from an ethanol production plant in Brazil. Distinctive phenotypic characteristics are the ability to metabolise sucrose but not trehalose (Badotti et al. 2015). The usage of glucose, cellobiose, trehalose, and mannose was demonstrated (Jamal et al. 2013).

O. oeni can grow at pH 3.0 and 10% ethanol. Many strains of *O. oeni* can even grow at 14% of ethanol (Bordas et al. 2013). Heat shock proteins and special membrane lipids are produced under these environmental conditions (Coucheney et al. 2005). Changes in the expression level of the geranylgeranyl pyrophosphate synthase gene was detected under ethanol stress (Cafaro et al. 2014b). Vigentini et al. (2016) isolated *O. oeni* strains from wineries of the Aosta Valley developing at 10 °C in Petit Rouge wine. These strains can be used for performing malolactic acid fermentation (MLF) in cold climate territories.

The DNA homology with other lactic acid genera is relatively low with a certain relationship to the genera *Leuconostoc* and *Weissella* (Stiles and Holzapfel 1997). The distinct pylogenetic position (Fig. 1.1) because of the quite different 16S rDNA sequence may indicate a quick evolving rRNA in the genus *Oenococcus* (Yang and Woese 1989), which could not be approved by a comparison of the gene sequences

of the DNA-dependent RNA-polymerases (Morse et al. 1996). Oenococci can be distinguished from less acid tolerant *Leuconostoc* species by using saccharose, lactose and maltose as substrate (Garvie 1986a).

It is important to use selected strains for wine making under special conditions, because some features are expressed at strain level. Insertion sequences (IS) could be one of the reasons for genotypic and phenotypic variants of oenococci (El Gharniti et al. 2012). The whole genome of different strains of *O. oeni* was performed, which allowed to define the invariant and variable DNA regions between the strains. Genetic variation in amino acid and sugar metabolism was a common feature (Capozzi et al. 2014; Lamontanara et al. 2014; Sternes and Borneman 2016). Protein expression profiling of *Oenococcus oeni* from Aglianico wine allowed to analyze the cellular pathways (Cafaro et al. 2014a). Mohedano et al. (2014) identified 152 unique proteins were identified *in O. oeni*.

O. oeni can use the hexoses glucose and fructose, while not all strains use trehalose (Garvie 1986a). L-arginine can be degraded to carbon dioxide, ammonia and ornithine. *O. oeni* can perform a malolactic fermentation (Caspritz and Radler 1983), which is also found in the genera *Lactobacillus*, *Leuconostoc*, and *Pediococcus*. The malolactic fermentation leads to a membrane potential and a proton gradient. With the aid of an F_1F_0 ATPase energy can be gained (Poolman et al. 1991).

Oenococci were able to synthesize capsular heteropolysaccharides made of glucose, galactose and rhamnose, β-glucans and homopolysaccharide (α-glucan or β-fructan) (Dimopoulou et al. 2014)

Oenococci exhibit a high mutability due to the lack of the mismatch repair genes *mutS* and *mutL* (Marcobal et al. 2008), which may facilitate the formation of strains. Specific methods for the rapid detection or differentiation of *O. oeni* strains in must and wine samples have been developed (Kelly et al. 1993; Viti et al. 1996; Zavaleta et al. 1997; Fröhlich 2002; Fröhlich and König 2004; Larisika et al. 2008).

O. oeni strains can contain bacteriophages (Doria et al. 2013; Jaomanjaka et al. 2013) and plasmides (Favier et al. 2012).

The type species is *O. oeni* DSM 20252^T.

O. oeni
Morphology: Spherical, lenticular cells in pairs or chains.
Characteristics: Growth below pH 3.0 and 10% ethanol.
Isolation: must/wine.
Type strain: DSM 20252^T.

1.7.4 Genus Pediococcus

Pediococci occur on plant material, fruits and in fermented food. They are non-pathogenic to plants and animals. Cells are spherical and never elongated as it is the case with leuconostocs and oenococci. The cell size is 0.36–1.43 μm in diameter.

Cell division occurs in two directions in a single plane. Short chains by pairs of cells or tetrads are formed (Garvie 1986b). Tetrad-forming homofermentative LABs in wine are pediococci. Pediococci are nonmotile and do not form spores or capsules (Simpson and Tachuchi 1995). The murein belongs to type A with an interpeptide bridge consisting of L-Lys-Ala-Asp (Holzapfel et al. 2003).

Glucose is fermented by the Embden–Meyerhof–Parnas pathway to DL or L-lactate. A wide range of carbohydrates is used such as hexoses, pentoses, disaccharides, trisaccharides and polymers such as starch. All wine-related species grow only in the presence of carbohydrates. The PTS system is used for glucose transport. Species producing DL-lactate possess an L- and D-LDH. Pyruvate can be converted mainly by *P. damnosus* to acetoin/diacetyl. *P. pentosaceus* and *P. damnosus* can degrade malate. They are nonproteolytic and nitrate is not reduced. Pediococci are catalase negative. Some strains of *P. pentosaceus* produce pseudocatalase. Pediococci do not reduce nitrate.

The G+C content of the DNA ranges from 34 to 44 mol%.

Pediococci can have plasmids, which code for production of bacteriocins or fermentation of carbohydrates. *P. pentosaceus* has three different plasmids for the fermentation of raffinose, melibiose and sucrose.

Pediococci are involved in beer spoilage (*P. damnosus*) and cause off-flavour in wine by production of diacetyl. *P. halophilus*, which has not been found in must/wine, is used to prepare soya sauce. Pediococci are used as starter culture in cheese production, silage and sausage production (*P. acidilactici*; *P. pentosaceus*). They play a role in cheese ripening. Pediococci (*P. acidilactici*; *P. pentosaceus*) can produce bacteriocins (pediocin) which can prevent meat spoilage. *P. damnosus* is a major spoilage organism in beer manufacturing, since it may produce diacetyl resulting in a buttery taste.

The species are differentiated by their range of sugar fermentation, hydrolysis of arginine, growth at different pH levels (4.5, 7.0), the configuration of lactic acid produced (Axelsson 2004) and ribotyping (Satokari et al. 2000). *P. pentosaceus* produces a nonheme pseudocatalase (Engesser and Hammes 1994).

The genus *Pediococcus* contains 11 species (July 2016; DSMZ 2016a). Four species have been found in must or wine (*P. damnosus*, *P. inopinatus*, *P. parvulus*, *P. pentosaceus*). Some characteristics of the species are compiled in Table 1.5).

The type species is *P. damnosus* DSM 20331^T.

P. damnosus
Morphology: Tetrades.
Characteristics: Ribose not fermented, arginine not hydrolysed. No growth at pH 8 or 35 °C. DL-lactic acid produced from glucose.
Isolation: Beer and wine.
Type strain: DSM 20331

P. inopinatus
Morphology: Tetrades
Characteristics: *P. parvulus* and *P. inopinatus* can be distinguished by the electrophoretic mobility of the L- and D-LDHs.

Isolation: Fermenting vegetables, beer, wine.
Type strain: DSM 20285

P. parvulus
Morphology: Tetrades, 0.7 μm × 1.1 μm in diameter. Single, pairs, tetrads, irregular clusters.
Characteristics: Grows at pH 4.5. Lactose, starch and pentoses not utilized. Arginine not hydrolysed. DL-lactic acid produced from glucose.
Isolation: Plant material, sauerkraut, fermented vegetables, fermented beans, beer, cider and wine.
Type strain: DSM 20332

P. pentosaceus
Morphology: Tetrades.
Characteristics: Pentoses and maltose fermented. Arginine is hydrolysed. Growth up to 45 °C. Used for the inoculation of semi-dry sausage, cucumber, green bean or soya milk fermentations and silage. Some strains produce pediocins. Isolation: Plant material and wine.
Type strain: DSM 20336

1.7.5 Genus Weissella

Based on rDNA analysis *Lc. paramesenteroides* ("*Lc. paramesenteroides* group") was reclassified as *W. paramesenteroides*. Five *heterofermentative lactobacilli* (*Lb. confusus*, *Lb. halotolerans*, *Lb. kandleri*, *Lb. minor*, *Lb. viridescens)* were also assigned to the genus *Weissella* (Collins et al. 1993; Björkroth and Holzapfel 2006). Weissellas are spherical, lenticular or irregular rods. They are heterofermentative species, which produce D, L-lactic acid, while *W. paramesenteroides* forms D-lactic acid from glucose. They have been isolated from food and meat. Weissellas produce greenish oxidized porphyrins in meat products by H_2O_2 accumulation. The genus *Weissella* contained 21 validly described species (July 2016, DSMZ 2016a). *W. paramesenteroides* is the only species of this genus isolated from must/wine.

The type species is *W. viridescens* DSM 20410^T.

W. paramesenteroides
Morphology: Sperical, lenticular
Characteristics: Pseudocatalase may be produced in the presence of low glucose content.
Isolation: must/wine, fresh vegetables, sausages
Type strain: DSM 20288^T

1.8 Conclusions

Lactic acid bacteria are widespread in habitats with complex nutritional supply such as plant material or fruit juice as well as animals. They influence the aroma, the quality, the consistency and safety of food. Since the 1900s, the production of fermented food and consequently the demand for starter cultures of lactic acid bacteria has been largely increased (Mäyrä-Mäkinen and Bigret 2004). They play an important role in the fermentation of sugar-containing food. Because of the acid formation and production of inhibitory components, they contribute to the preservation of food. On the other hand, they can produce off-flavour (e.g. diacetyl) and cause ropiness by exopolysaccharide production.

Especially in northern wine growing regions, grapes can contain high amounts of acid with unfavourable organoleptic properties. So far, mainly *O. oeni* and sometimes *Lb. plantarum* are used as starter cultures for wine making to reduce the malic acid content.

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