CHAPTER 2

# LACTIC ACID BACTERIA

## 2.1 INTRODUCTION

Lactic acid bacteria comprise an ecologically diverse group of microorganisms united by formation of lactic acid as the primary metabolite of sugar metabolism (Davis et al., 1985b; 1988; Lonvaud-Funel, 1999; Carr et al., 2002; Liu, 2002). These bacteria utilize sugars by either homo- or heterofermentative pathways (Section 2.4.1) as well as L-malic acid, a major acid present in grape must (Section 2.4.3). Whereas growth of some bacteria in certain wines is desirable (i.e., malolactic fermentation or MLF), growth of other species can lead to spoilage.

## 2.2 TAXONOMY

The lactic acid bacteria isolated from grape musts or wine belongs to two families representing three genera. The Lactobacillaceae are represented by the genus *Lactobacillus*, and the Streptococcaceae are represented by the genera *Oenococcus* and *Pediococcus*.

### 2.2.1 *Lactobacillus*

*Lactobacillus* represents a highly diverse group of Gram-positive, microaerophilic bacteria that microscopically appear as long to short rods or even coccobacilli (Fig. 2.1) (Kandler and Weiss, 1986). Species within this genus are generally catalase-negative, although a few strains decompose peroxide by a non-heme-containing pseudo-catalase (Johnston and Delwiche, 1962; Kono and Fridovich, 1983; Beyer and Fridovich, 1985). *Lactobacillus* spp. are either homo- or heterofermentative with regard to hexose metabolism (Section 2.4.1). Physiological characteristics used to identify some species of *Lactobacillus* found in grape musts or wines are presented in Table 2.1.

Various species of *Lactobacillus* that have been isolated from grapes and wines worldwide including *L. brevis*, *L. buchneri*, *L. casei*, *L. cellobiosus*, *L. curvatus*, *L. delbrueckii*, *L. diolivorans*, *L. fructivorans*, *L. heterohiochii*, *L. hilgardii*, *L. jensenii*, *L. kunkeei*, *L. leichmanni*, *L. nagelli*, *L. paracasei*, *L. plantarum*, *L. trichodes*, *L. vermiforme*, and *L. yamanashiensis* (Douglas and Cruess, 1936; Vaughn, 1955; Fornachon, 1957; Kitahara et al., 1957,

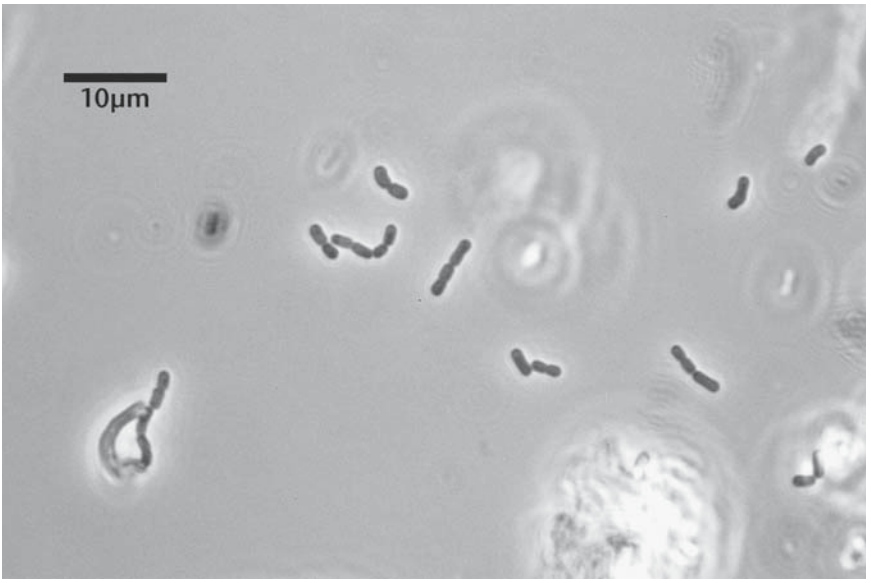


Figure 2.1. *Lactobacillus brevis* as viewed with phase-contrast microscopy at a magnification of 1000×. Photograph provided with the kind permission of WineBugs LLC.

**Table 2.1. Characteristics of some *Lactobacillus* found in wines.**

| Characteristic | *L. brevis* | *L. hilgardii* | *L. kunkeei* | *L. plantarum* |
|---|---|---|---|---|
| Ammonia from arginine | + | + | − | − |
| Catalase | v | + | w | v |
| Gas from glucose | + | + | + | − |
| Hydrolysis of esculin | v | − | − | + |
| Lactic acid from glucose | DL | DL | L | DL |
| Mannitol from fructose | + | + | + | − |
| Fermentation of: | | | | |
| Arabinose | + | − | − | v |
| Fructose | + | + | + | + |
| Lactose | v | v | − | + |
| Mannitol | − | − | + | + |
| Maltose | + | + | − | + |
| Melezitose | − | v | − | + |
| Ribose | + | + | − | + |
| Sucrose | v | v | + | + |
| Trehalose | − | − | − | + |
| Xylose | v | + | − | v |

(+) 90% or more of the strains are positive; (−) 90% or more of the strains are negative; (v) variable response of strains; (w) weak reaction.
Data from Kandler and Weiss (1986), Dicks and van Vuuren (1988), Pilone et al. (1991), Hammes et al. (1992), and Edwards et al. (1993; 1998).

Du Plessis and van Zyl, 1963a; Pilone et al., 1966; Carr et al., 1977; Chalfan et al., 1977; Maret and Sozzi, 1977; 1979; Costello et al., 1983, Lafon-Lafourcade et al., 1983b; Nonomura, 1983; Davis et al., 1986a; 1986b; Dicks and van Vuuren, 1988; Sieiro et al., 1990; Edwards et al. 1993; 1998a; 2000; Mills, 2001; Gorga et al., 2002; Beneduce et al., 2004; Du Plessis et al., 2004).

Recent evidence has resulted in changes in the taxonomy of the lactobacilli. Reflecting this, *L. cellobiosus* is currently regarded as a synonym of *L. fermentum*, and *L. leichmanni* is now referred to as *L. delbrueckii* subsp. *lactis* (Kandler and Weiss, 1986). *L. trichodes* and *L. heterohiochii* (Kitahara et al., 1957) are now considered synonyms of *L. fructivorans* (Weiss et al., 1983). Edwards et al. (1998a; 2000) isolated two novel *Lactobacillus* spp. from commercial grape wines undergoing sluggish/stuck alcoholic fermentations. Based on phenotypic and phylogenetic evidence, *L. kunkeei* and *L. nagelii* were proposed as new species. Few reports are available describing *L. vermiforme* (Sharpe et al., 1972; Garvie, 1976), and it is not clear whether the bacterium represents a separate species or is a synonym of a closely related species, *L. hilgardii*.

### 2.2.2 *Oenococcus*

Wine bacteria belonging to the genus *Oenococcus* have been previously classified as *Leuconostoc gracile*, *Leuconostoc citrovorum*, and *Leuconostoc oenos* (Pilone and Kunkee, 1965; Garvie, 1967a; Kunkee, 1967a). Later phylogenetic studies revealed that *L. oenos* represented a distinct subline separate from other *Leuconostoc* spp. (Martinez-Murcia et al., 1993), a finding that resulted in reassignment of this bacterium to a new genus, *Oenococcus* (Dicks et al., 1995). Given the diversity in physiological characteristics such as carbohydrate fermentation patterns, Tracey and Britz (1987) suggested that it is possible that *O. oeni* could represent more than one species.

Strains of *O. oeni* are described as Gram-positive, nonmotile, facultatively anaerobic, catalase-negative, ellipsoidal to spherical cells that usually occur in pairs or chains (Fig. 2.2) (Garvie, 1967a; 1986a; Holzapfel and Schillinger, 1992; Dicks et al., 1995). Cells can be difficult to differentiate microscopically from short rods of Lactobacillus (Fig. 2.1). The species is heterofermentative, converting glucose to equimolar amounts of primarily D-lactic acid, $CO_2$, and ethanol or acetate (Krieger et al., 1993; Cogan and Jordan, 1994; Cocaign-Bousquet et al., 1996). The bacterium produces gas from glucose, hydrolyzes esculin, forms D-lactic acid from glucose and

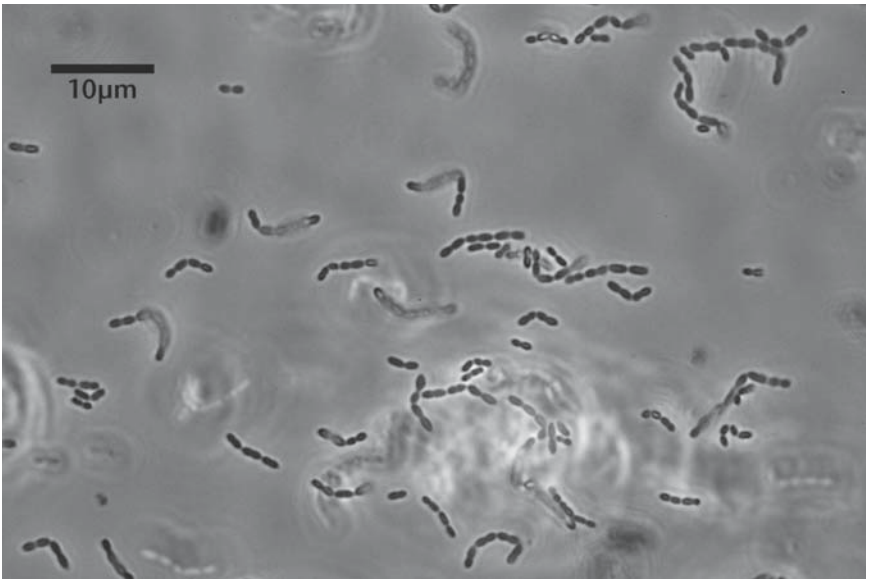


Figure 2.2. *Oenococcus oeni* as viewed with phase-contrast microscopy at a magnification of 1000×. Photograph provided with the kind permission of WineBugs LLC.

mannitol from fructose, and may produce ammonia from arginine (Pilone et al., 1991; Holzapfel and Schillinger, 1992; Dicks et al., 1995).

Although only one species is assigned to this genus, *O. oeni* belongs to a heterogeneous group evidenced by wide variability in the fermentation of specific carbohydrates (Lafon-Lafourcade et al., 1983b; Tracey and Britz, 1987; Davis et al., 1988; Kelly et al., 1989; Edwards et al., 1991). Most strains of *O. oeni* utilize L-arabinose, fructose, and ribose but not galactose, lactose, maltose, melezitose, raffinose, or xylose. By comparison, Lafon-Lafourcade et al. (1983b) noted that only 11% of the strains evaluated in their study utilized both fructose and glucose, contrary to the findings of others (Pilone and Kunkee, 1972; Beelman et al., 1977; Izugabe et al., 1985; Edwards et al., 1991). Davis et al. (1988) determined that only 55% of the strains studied fermented ribose, 27% fermented D-arabinose, and 45% fermented sucrose. Strain A-9 described by Chalfan et al. (1977) fermented glucose but not fructose. Although discrepancies in carbohydrate fermentations are probably the result of strain characteristics, differences in techniques used to determine carbohydrate fermentability (Pardo et al., 1988; Jensen and Edwards, 1991) and the nutritional composition of media given the fastidious nature of *Oenococcus* (Garvie, 1967a; 1967b) may also cause variable results.

*O. oeni* has the ability to metabolize malic acid found in grapes to form lactic acid through MLF (Sections 2.4.3 and 6.4.2). Though other species of lactic acid bacteria have been investigated and used as commercial starters for MLF, strains of *O. oeni* appear to have the physiological properties to consistently tolerate the environmental challenges of wine while producing desirable results within an amount of time acceptable to the winemaker.

### 2.2.3 *Pediococcus*

Of the approved species of *Pediococcus* (Garvie, 1986b; Weiss, 1992), only four have been isolated from wines; *P. damnosus*, *P. parvulus*, *P. inopinatus*, and *P. pentosaceus* (Davis et al., 1986a; 1986b; Edwards and Jensen, 1992). Several researchers previously reported isolation of *P. cerevisiae* from wines (Maret and Sozzi, 1977; 1979; Costello et al., 1983; Lafon-Lafourcade et al., 1983b; Fleet et al., 1984). The species is now considered invalid because it represents at least two species including *P. damnosus* and *P. pentosaceus* (Garvie, 1974; Raccach, 1987). *P. damnosus* and *P. parvulus* appear to be more commonly found in wines than the other species.

Pediococci are characterized as being Gram-positive, nonmotile, catalase-negative, and aerobic to microaerophilic bacteria (Garvie, 1986b; Pilone et al., 1991; Weiss, 1992). Members of this genus are

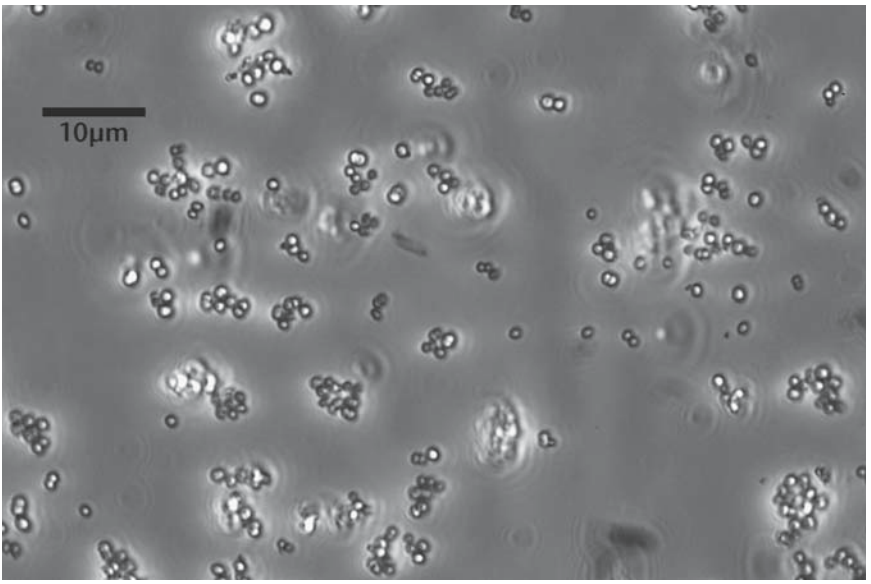


Figure 2.3. *Pediococcus damnosus* as viewed with phase-contrast microscopy at a magnification of 1000×. Photograph provided with the kind permission of WineBugs LLC.

homofermentative (Section 2.4.1), with glucose converted to either L- or DL-lactate (Garvie, 1986b). Under glucose limitation, Pasteris and Strasser de Saad (2005) noted that a strain of *P. pentosaceus* degraded glycerol to pyruvate, the latter being further metabolized to either acetate or diacetyl or 2,3-butanediol through "active-acetaldehyde" (Section 2.4.5). Growing cultures commonly possess the ability to form L-lactate from L-malic acid (Raccach, 1987; Edwards and Jensen, 1992). Pediococci are chemoorganotrophs and require complex growth factor and amino acid requirements. In addition, these are the only lactic acid bacteria that divide in two planes, which results in the formation of pairs, tetrads or large clumps of spherical cells as shown in Fig. 2.3 (Garvie, 1986b; Axelsson, 1998). Characteristics for three species of *Pediococcus* are listed in Table 2.2.

## 2.3 NUTRITIONAL REQUIREMENTS

Lactic acid bacteria have very limited biosynthetic capabilities and, reflecting this, are described as nutritionally fastidious. Early work by Du Plessis (1963) noted that all strains of wine lactic acid bacteria required nicotinic acid, riboflavin, pantothenic acid, and either thiamine or pyridoxine.

**Table 2.2. Characteristics of *Pediococcus*.**

| Characteristic | *P. damnosus* | *P. parvulus* | *P. pentosaceus* |
|---|---|---|---|
| Ammonia from arginine | − | − | + |
| Catalase | − | − | v |
| Gas from glucose | − | − | − |
| Hydrolysis of esculin | + | + | + |
| Lactic acid from glucose | DL | DL | DL |
| Mannitol from fructose | − | − | − |
| Fermentation of: | | | |
| Arabinose | − | − | + |
| Fructose | + | + | + |
| Lactose | − | − | v |
| Mannitol | − | − | − |
| Maltose | v | + | + |
| Melezitose | v | − | − |
| Ribose | − | − | + |
| Sucrose | v | − | − |
| Trehalose | + | v | + |
| Xylose | − | − | v |

(+) 90% or more of the strains are positive; (−) 90% or more of the strains are negative; (v) variable response of strains.
Data from Garvie (1986b), Pilone et al. (1991), and Weiss (1992).

More recently, Garvie (1986b) reported that all species of *Pediococcus* required nicotinic acid, pantothenic acid, and biotin, whereas none required thiamine, *p*-aminobenzoic acid, or cobalamine. Several amino acids (glutamic acid, valine, arginine, leucine, and isoleucine) appear to be essential for growth. Garvie (1967b) reported similar results but included cysteine, tyrosine, and others depending on the strain of *Oenococcus* (*Leuconostoc*). In addition, purines (guanine, adenine, xanthine, and uracil) and folic acid are also required by many species. Finally, it should be noted that lactic acid bacteria cannot utilize diammonium phosphate as a nitrogen source and so must rely on amino acids.

Another important nutrient is the so-called tomato juice factor (Garvie and Mabbitt, 1967). This nutrient was named for the fact that many lactic acid bacteria isolated from grape musts or wines seemed to grow better on media supplemented with either fruit or vegetable juices or serums such as tomato or apple (Section 13.6). This requirement varies with growth conditions and strains (Garvie, 1984). In fact, Tracey and Britz (1987) were able to grow a number of strains of *O. oeni* in the absence of the tomato juice factor, although growth was much slower. Amachi (1975) ascertained its structure to be a derivative of pantothenic acid, 4-O-(α-D-glucopyranosyl)-D-pantothenic acid.

## 2.4 METABOLISM

### 2.4.1 Glucose

After completion of alcoholic fermentation, low concentrations of hexose sugars may remain in the wine. These include glucose and fructose with lesser amounts of mannose and galactose. Among the five-carbon sugars (pentoses), arabinose, ribose, and xylose are the most common. Further, there may be sufficient quantities of sugar to support the growth of lactic acid bacteria in "dry" wines.

Lactic acid bacteria utilize sugars (e.g., glucose) to form lactic acid by either the homo- or heterofermentative pathway. The homofermentative pathway, illustrated in Fig. 2.4, results in the transformation of glucose to pyruvate through the Embden–Meyerhof–Parnas pathway (EMP, or glycolysis), eventually yielding lactic acid. NADH produced by the oxidation of glyceraldehyde-3-phosphate to 1,3-bisphosphoglycerate is reoxidized to $NAD^+$ in the formation of lactate from pyruvate through the action of lactate dehydrogenases (LDHs). The LDH enzymes vary in their stereospecificity and can yield D- or L-lactic acid or the racemic mixture (DL).

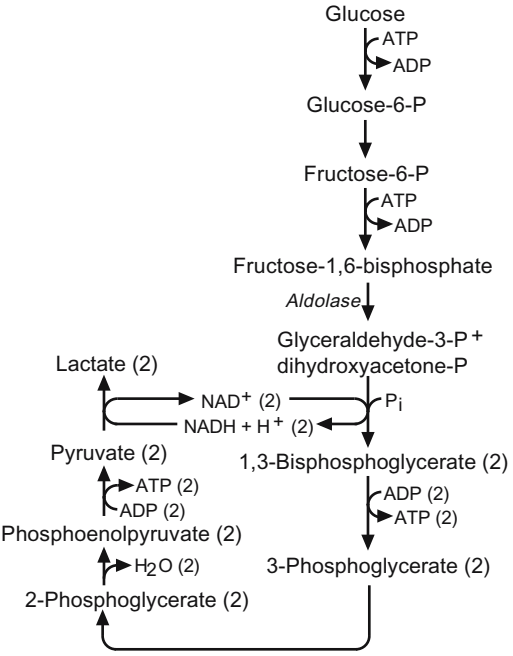


Figure 2.4. Homofermentative pathway illustrating the production of lactic acid.

Though 1 mole of glucose should produce 2 moles of lactic acid, the actual yield is closer to 1.8 moles of lactic acid (Gottshalk, 1986). Energetically, glycolysis yields 2 moles ATP per mole glucose. The diagnostic enzyme present in those microorganisms that possess this pathway, aldolase, catalyzes the conversion of 1 mole of fructose-1,6-bisphosphate to 2 moles of glyceraldehyde-3-phosphate (Fig. 2.4). As was the case with *Saccharomyces*, these bacteria cannot metabolize pentoses. Examples of lactobacilli that are obligate homofermenters are *L. delbrueckii* and *L. jensenii*.

Obligate heterofermenters (e.g., *O. oeni*, *L. brevis*, *L. hilgardii*, *L. fructivorans*, and *L. kunkeei*) lack aldolase and must divert the flow of carbon through a different series of reactions, the pentose phosphate, or phosphoketolase, pathway (Fig. 2.5). From 1 mole of glucose, heterofermentative bacteria produce 1 mole each of lactate, $CO_2$, and either acetic acid or ethanol. In reality, these bacteria produce 0.8 mole lactate from glucose (Gottshalk, 1986). Unlike homofermentative microorganisms, these bacteria do not have aldolase but possess phosphoketolase, the

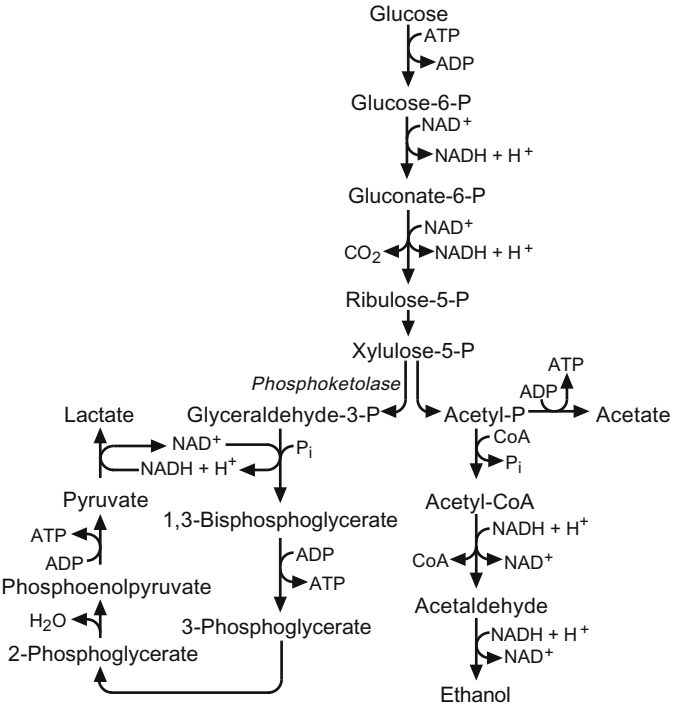


Figure 2.5. Heterofermentative pathway showing production of lactic acid, $CO_2$, and either ethanol or acetic acid.

enzyme responsible for the cleavage of xylulose-5-phosphate to form glyceraldehyde-3-phosphate and acetyl phosphate. Due to the biosynthesis of five-carbon sugars in this pathway (ribulose-5-phosphate and xylulose-5-phosphate), some strains can utilize the pentoses present in wine such as ribose, xylose, and arabinose. An important consequence of only half of the carbon from glucose going to glyceraldehyde-3-phosphate is formation of only 1 mole of ATP per mole glucose. However, heterofermentative bacteria can gain additional energy though conversion of acetyl-phosphate to acetate (Fig. 2.5).

From the winemaker's perspective, Fig. 2.5 highlights an important facet of successful management of these bacteria. Specifically, acetic acid production can result from conversion of both hexose and pentoses under even slight oxidative conditions. Under reductive conditions, cells experience a shortage of $NAD^+$ and so acetyl phosphate is converted to ethanol rather than acetate. Conversely, acetyl phosphate can be used to produce energy (ATP) under oxidative conditions in formation of acetate and increased volatile acidity (Section 11.3.1).

Besides obligate homo- and heterofermentative bacteria, Kandler and Weiss (1986) also described a third group of bacteria known as the facultative heterofermenters. Although these bacteria utilize hexoses through the homofermentative pathway (Fig. 2.4), they also possess an inducible phosphoketolase with pentoses acting as inducers (Fig. 2.5). Examples of wine bacteria belonging to this group are *L. casei* and *L. plantarum*.

## 2.4.2 Arginine

Many heterofermentative lactic acid bacteria have the ability to produce energy through the utilization of arginine in formation of ornithine, $NH_3$, $CO_2$, and ATP (Fig. 2.6). The ability of lactic acid bacteria to produce ammonia from arginine can be determined using the method outlined in Section 15.4.1.

It has been thought that most heterofermentative lactobacilli produce $NH_3$ from arginine, whereas homofermentative lactobacilli and *O. oeni* do not (Garvie, 1967a; Kandler and Weiss, 1986; Tonon and Lonvaud-Funel, 2002). However, Pilone et al. (1991) questioned the sensitivity of Nessler's reagent commonly used to detect the low concentrations of ammonia produced. Furthermore, Pilone et al. (1991) suggested that some heterofermentative lactobacilli are capable of carrying out only the first two biochemical steps, thus only yielding one molecule of $NH_3$ per molecule of arginine (Fig. 2.6). Because of these problems, the authors recommended that the concentration of the amino acid be increased from 0.3% to 0.6% w/v. Using an increased concentration of L-arginine, Pilone et al.

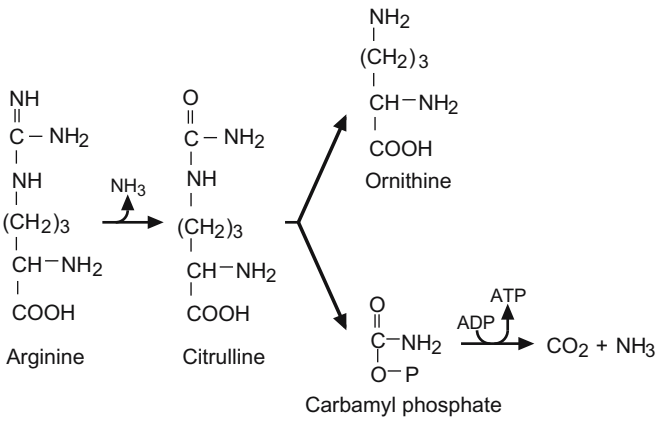


Figure 2.6. Formation of ornithine, ammonia, and carbon dioxide from arginine by some heterofermentative lactic acid bacteria.

(1991) found that some strains of *O. oeni* did, in fact, produce ammonia. In addition to problems related to concentration of arginine, Liu et al. (1995b) noted that fructose is inhibitory to arginine degradation by some bacterial strains.

### 2.4.3 Malate

Although malic acid stimulates the growth of *O. oeni* (Firme et al., 1994), the biochemical benefit of MLF to the bacterium has been a mystery because formation ATP or other direct energy could not be detected (Pilone and Kunkee, 1972). This prompted researchers to suggest that the reaction must serve a non-energy-yielding function (Kunkee, 1967b; Pilone and Kunkee, 1972). However, it became clear that MLF does, in fact, produce energy in an indirect means based on the chemiosmotic theory (Gottschalk, 1986), which holds that viable microorganisms maintain a pH gradient across cell membranes and it is this gradient that allows energy (ATP) to be produced. Under normal conditions, a higher concentration of $H^+$ exists outside compared with the interior of the cell. As a proton ($H^+$) travels through a membrane-associated enzyme complex (ATPase) following the concentration gradient from high to low concentration, this allows the bacterium to generate one molecule of ATP from ADP and inorganic phosphate ($P_i$). The model described requires that the membrane be impermeable to protons except at specific sites where the ATPase complex is located.

Cox and Henick-Kling (1989; 1995) were able to demonstrate that MLF yielded ATP and proposed that the ability of a cell to expel lactate and

protons could theoretically generate a proton motive gradient that, in turn, would yield ATP through the ATPase. A variation of this model was proposed (Poolman et al., 1991; Salema et al., 1994) in which L-malate is taken up in the monoanionic form (the dominant species at low pH) as illustrated in Fig. 2.7. This would cause a net negative charge to be moved into the cell and thereby create an electrical potential. L-Malate is then decarboxylated to L-lactic acid and $CO_2$ in a reaction that requires one proton. The consumption of a proton in the cytoplasm generates a pH gradient that, together with the change in electrical potential, allows ATP generation to occur by a membrane-bound ATPase. Salema et al. (1994; 1996) suggested that L-lactic acid and $CO_2$ leave the cell as neutral species rather then being actively transported across the membrane.

## 2.4.4 Mannitol and Erythritol

As stated previously, many heterofermentative lactic acid bacteria gain additional energy by converting acetyl phosphate to acetate instead of ethanol. Although an additional ATP can be produced, the cell requires regeneration of $NAD^+$, a process achieved using an alternative electron acceptor, fructose (Wisselink et al., 2002). The reduction of fructose to mannitol by lactic acid bacteria catalyzed by mannitol dehydrogenase is shown in Fig. 2.8.

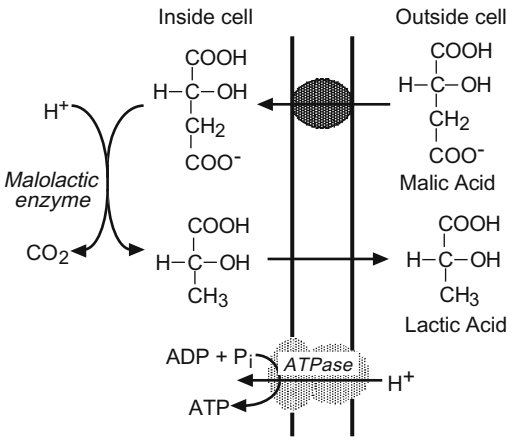


Figure 2.7. Proposed model for energy generation (ATP) by *Oenococcus oeni* through conversion of malic acid to lactic acid and carbon dioxide. Modified from Poolman et al. (1991) with the kind permission of the *Journal of Bacteriology*.

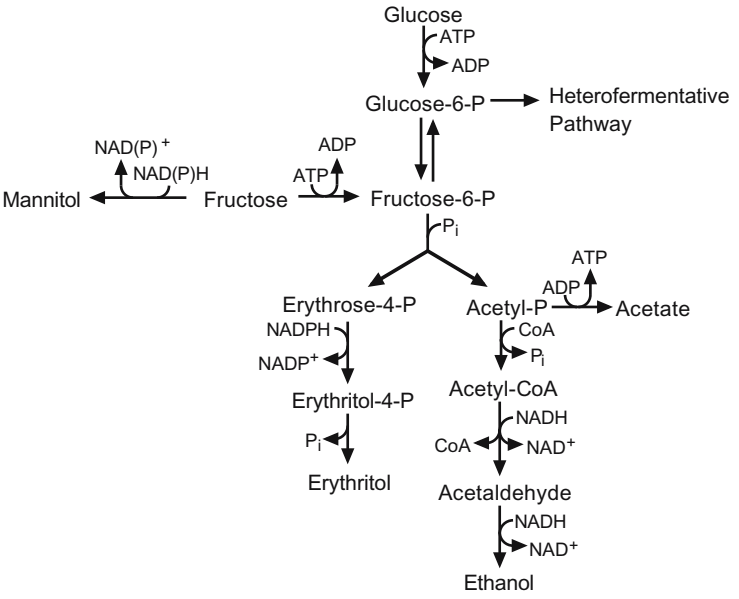


Figure 2.8. Formation of mannitol and erythritol. Adapted from Veiga-da-Cunha et al. (1993) with the kind permission of the *Journal of Bacteriology*.

Mannitol formation is used as a laboratory diagnostic test for the separation of heterofermentative from homofermentative bacteria (Section 15.4.10). Although primarily a property of heterofermentative lactic acid bacteria, a few homofermentative strains can also produce small amounts of the sugar alcohol (Wisselink et al., 2002).

Veiga-da-Cunha et al. (1993) observed that *O. oeni* produced another sugar alcohol, erythritol, anaerobically from glucose but not from fructose or ribose. In the presence of oxygen, synthesis of this sugar alcohol was absent. In agreement, Firme et al. (1994) reported erythritol production by this bacterium under $N_2$ or $CO_2$ environments. As with the formation of mannitol, synthesis of erythritol is probably related to the cell's need to reoxidize NADPH under anaerobic conditions.

## 2.4.5 Diacetyl and Other Odor/Flavor Compounds

One of the most important odor active compounds produced by lactic acid bacteria is 2,3-butandione, or diacetyl (Fornachon and Lloyd, 1965; Collins, 1972; El-Gendy et al., 1983; Rodriguez et al., 1990; Martineau and

Henick-Kling, 1995a; 1995b; Nielsen and Richelieu, 1999; Bartowsky and Henschke, 2004a; 2004b). Diacetyl has a distinct "buttery" aroma with a sensory threshold ranging from 0.2 mg/L in Chardonnay to 2.8 mg/L in Cabernet Sauvignon wines (Martineau et al., 1995). Whereas the presence of diacetyl at low concentrations (1 to 3 mg/L) is described sensorially as being "buttery" or "nutty," the compound will dominate the aroma profiles at concentrations greater than 5 to 7 mg/L, potentially resulting in spoilage (Rankine et al., 1969). Perception of the "buttery" aroma cannot always be predicted directly from diacetyl concentrations due to differences in matrix and other factors (Bartowsky et al., 2002).

Diacetyl may be synthesized by either homolactic or heterolactic pathways of sugar metabolism as well as by utilization of citric acid (Fig. 2.9). Citric acid is first converted to acetic acid and oxaloacetate; the latter is then decarboxylated to pyruvate. Although earlier reports indicated that diacetyl synthesis by lactic acid bacteria does not proceed via α-acetolactate (Gottschalk, 1986), more recent evidence suggests that this pathway is active in lactic acid bacteria (Ramos et al., 1995). Here, pyruvate undergoes a second decarboxylation and condensation with thiamine pyrophosphate (TPP) to yield "active acetaldehyde." This compound then reacts with another molecule of pyruvate to yield α-acetolactate, which, in

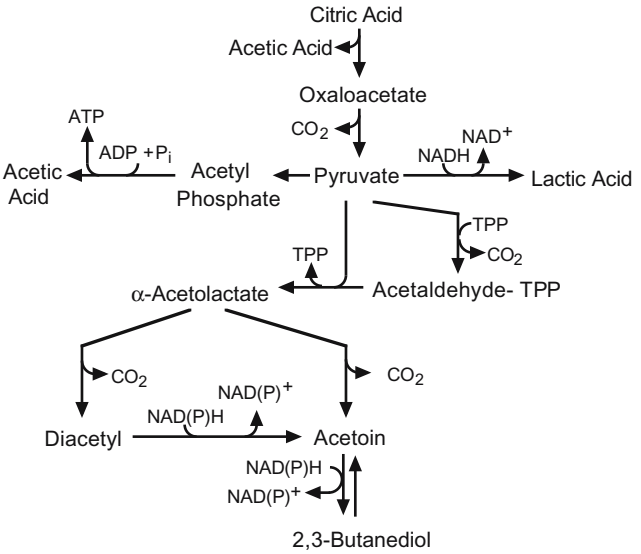


Figure 2.9. Biochemical formation of diacetyl, acetoin, and 2,3-butanediol by lactic acid bacteria. Adapted from Ramos et al. (1995), Bartowsky and Henschke (2004b), and Ribereau-Gayon et al. (2000). TPP refers to thiamine pyrophosphate.

turn, undergoes oxidative decarboxylation to produce diacetyl (Ramos et al., 1995; Bartowsky and Henschke, 2004b). Diacetyl can be further transformed into acetoin as well as 2,3-butanediol. An alternate pathway involving the reaction of "active acetaldehyde" with acetyl CoA has been proposed, but the responsible enzyme, diacetyl synthetase, has never been isolated (Ribéreau-Gayon et al., 2000).

During growth, malic and citric acid utilization by lactic acid bacteria may occur concomitantly, although utilization of citric acid proceeds at a much slower rate (Pimentel et al., 1994). Thus, complete conversion of citric acid does not necessarily coincide with completion of MLF, and levels of citric acid remaining in the wine post-MLF may be sufficient to stimulate bacterial formation of diacetyl and acetic acid.

Microbial formation of diacetyl is a dynamic process, and concentrations in the wine depend on many factors including bacterial strain, wine type, and redox potential (Martineau and Henick-Kling, 1995a; 1995b; Nielsen and Richelieu, 1999). For instance, MLF during or just after alcoholic fermentation, when high populations of yeast are present, yields lower amounts of diacetyl due to rapid yeast reduction to acetoin and butylene glycol. By comparison, MLF occurring in low-density populations of viable yeast results in correspondingly higher concentrations of diacetyl. In general, diacetyl levels produced by *O. oeni* are relatively low compared with *Lactobacillus* or *Pediococcus*, which can synthesize objectionable concentrations. Prahl and Nielsen (1995) illustrated that the reversible reaction between diacetyl and $SO_2$ can result in rapid decreases in the concentration of diacetyl from 30% to 60%. Because the reaction is transitory, however, objectionable levels may return after several weeks of storage. Factors that impact the synthesis of diacetyl by lactic acid bacteria are summarized in Table 2.3.

Besides diacetyl, *O. oeni* can also synthesize higher alcohols and other compounds as by-products of their metabolism (Tracey and Britz, 1989; Edwards and Peterson, 1994; De Revel et al., 1999; Maicas et al., 1999; Delaquis et al., 2000). More recent evidence indicates that *O. oeni* possesses β-glucosidase activity (Section 1.5.4), an enzyme responsible for hydrolysis of monoglucosides, which can alter the sensory characteristics of a wine (Grimaldi et al., 2000; Boido et al., 2002; Mansfield et al., 2002; Ugliano et al., 2003; D'Incecco et al., 2004).

Osborne et al. (2000) reported that *O. oeni* can metabolize acetaldehyde producing ethanol and acetic acid. In some cases, this may be desirable because excess acetaldehyde may result in wine spoilage (Kotseridis and Baumes, 2000; Liu and Pilone, 2000). However, acetaldehyde also plays a role in the color development and stabilization of red wines (Timberlake and Bridle, 1976). More recently, Morneau and Mira de Orduna (2005)

**Table 2.3. Factors that affect diacetyl synthesis by lactic acid bacteria.**

| Factor | Impact |
|---|---|
| Bacterium | • Synthesis varies with genus, species and strain. |
| Inoculation rate | • Lower initial inoculums ($10^4$ vs. $10^6$ CFU/mL) of bacteria favors synthesis. |
| Wine contact with yeast lees (*sur lies*) | • Conflicting studies where synthesis (Boulton et al., 1996) and degradation (Bartowsky and Henschke, 2004a) have been reported. |
| Wine contact with air | • Nonenzymatic reaction of α-acetolactate to diacetyl favored. |
| Addition of $SO_2$ | • Binds diacetyl yielding sensory inactive adduct.<br>• Inhibits bacteria |
| Addition of citric acid | • Favors synthesis (increases acetic acid too). |
| Temperature | • More diacetyl retained in wines undergoing MLF at 18°C than 25°C. |
| pH | • Lower pH retards growth of bacteria but favors synthesis. |

CFU, colony-forming units; MLF, malolactic fermentation.
Adapted from Bartowsky and Henschke (2004a) and Boulton et al. (1996).

demonstrated that acetaldehyde degradation was strain as well as pH and $SO_2$ dependent.

Although *O. oeni* produces a variety of volatile compounds, the debate continues regarding the exact contribution of malolactic fermentation to the sensory properties of a wine. Early work by Kunkee et al. (1964) and Rankine (1972) indicated that except for its role in deacidification, MLF did not have a measurable influence on sensory properties of wine. On the other hand, more recent studies suggested differential changes in wine aroma and flavor (McDaniel et al., 1987; Laurent et al., 1994; Henick-Kling, 1995; Sauvageot and Vivier, 1997; Nielsen and Richelieu, 1999; Delaquis et al., 2000; Gambaro et al., 2001; Boido et al., 2002). For example, Sauvageot and Vivier (1997) noted that Chardonnay wines that completed MLF were perceived as being higher in "hazelnut," "fresh bread," and "dried fruit" aromas, whereas Pinot noir lost "strawberry" and "raspberry" sensory notes. Aside from influencing flavor and aroma, MLF may increase the body and mouthfeel, possibly due to the production of polyols such as glycerol and erythritol (Henick-Kling et al., 1994). Pripis-Nicolau et al. (2004) determined that lactic acid bacteria could metabolize methionine to produce 3-(methylsulphanyl)propionic acid. The authors described this compound as "chocolate" and "roasted" and theorized that this acid could contribute to the sensory complexity of wines post-MLF.