Lactic Acid Bacteria and Wine Spoilage*

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Lactic acid bacteria (LAB) are responsible for many fermented foods such as sauerkraut, pickles and yogurt. They have also been isolated from wines at various states of vinification. In wines they are responsible for malolactic fermentation (MLF) which can be beneficial in some cases and undesirable in others. Besides conducting MLF, these bacteria under certain conditions can also cause undesirable changes in wine flavor which renders the wine undrinkable. Many species of LAB do not conduct MLF and their growth in wine can cause some serious wine spoilage.

Nature of Lactic Acid Bacteria

Lactic Acid bacteria found in wine belong to three genera, namely:

1. Leuconostoc - Heterofermentative cocci, oval or spherical, occur in pairs or chains.
2. Pediococcus - Homofermentative cocci, often found in tetrads.
3. Lactobacillus - Homofermentative or heterofermentative rods, found singly or in chains.

These organisms are gram positive, catalase negative, nonsporing cocci, coccobacilli or rods. They are microaerophilic that means they grow well under conditions of low oxygen content. Since they can grow under low oxygen conditions, they can grow throughout the wine (as opposed to on the surface of the wine) even though the container is kept full. The bacteria can metabolize sugars, acids and other constituents in wine and produce several compounds. Some of these are undesirable and constitute spoilage.

Source of Lactic Acid Bacteria

The bacteria can be found on the surface of grapes and grape leaves. During the harvest, the bacteria gain entry into the winery with the grapes. Their population on the surface of the fruit is generally low and it depends on the level of maturity and the condition of the fruit. Another source of these organisms in a winery is the contamination equipment. These may include pumps, valves and storage containers. Wooden barrels which are often difficult to clean and sanitize can be a source of these bacteria if the barrels contain MLF wine and were not properly cleaned.

Occurrence of Lactic Acid Bacteria at Various Stages of Vinification

At crush the bacterial population is small, about 103 to 104 cells/mL. Species belonging to genera Lactobacillus, Pediococcus and Leuconostoc have been found to occur at this stage. In the next stage, i.e. alcoholic fermentation, the population of these bacteria declines. This may be due to the competition by yeast and formation of ethanol and sulfur dioxide by the yeast during the alcoholic fermentation.

Following the alcoholic fermentation, the surviving bacteria grow vigorously and conduct MLF. The cell population can reach as high as 106 to 108 cells/mL. Usually species of Leuconostoc grow and conduct MLF but in the case of high pH wines (pH 3.5 and above), species of Pediococcus and Lactobacillus could be involved in MLF.

After MLF the fate of lactic acid bacteria depends on wine composition and how the wine is handled. If the wine pH is high (>3.5), and the SO_2 level is inadequate, then the spoilage causing species of LAB can grow and spoil the wine. For this reason special attention should paid to the wines during storage after MLF.

Nature of Spoilage by Lactic Acid Bacteria

The nature and the extent of wine spoilage by LAB depends on several factors such as the type of bacteria, composition of the wine and vinification practices. Based on the substrate used lactic spoilage has been classified as follows:

1. Fermentation of Sugars

LAB, including those involved in MLF, metabolize sugars such as glucose and fructose, and produce lactic acid and acetic acid. The resulting wine acquires a sour vinegar-like aroma due to high VA levels. This is a serious spoilage and occurs in must with stuck fermentation or wines with higher residual sugars (sweet wines).

A less serious form of lactic spoilage can occur in dry wines. In these wines the LAB utilizes pentose sugars, trace amounts of glucose and fructose, and produces lactic and acetic acid as a by-product. When sugars are attacked by LAB, lactic and acetic acids are produced. Formation of these acids increases the titratable acidity and lowers the pH. The decrease in pH restricts the growth of those organisms.

2. Degradation of Glycerol

Breakdown of glycerol by LAB results in the formation of lactic acid, acetic acid and acrolein. The wine smells acetic, butyric and acquires a bitter taste due to acrolein.

3. Fermentation of Tartaric Acid.
In this kind of spoilage, the LAB ferments tartaric acid and forms lactic acid, acetic acid and carbon dioxide. Degradation of tartaric acid occurs especially in wines with low acidity and high pH (pH above 3.5). The titratable acidity is further reduced and the wine acquires an acetic aroma and disagreeable taste. In advanced cases the wine is sometimes referred to as mousy.

4. Fermentation of Citric Acid
Citric acid content of a wine can decrease during MLF. Depending on the species of bacteria and the wine pH. Citric acid degradation has been positively correlated with the formation of diacetyl and acetone as well as acetic acid.

5. Ropiness
Certain species of Leuconostoc have been found to produce dextran slime or mucilaginous substances in wine. The wine appears oily and may not necessarily have high volatile acidity.

In general, the lactic acid bacteria (as a group) are involved in the fermentation of malic acid and other wine constituents. Their activity results in the formation of several components that impart off flavors to wine. Some of the terms used in describing the lactic spoilage in wine include: acetic or sour, buttery, cheesy, sauerkraut-like, bitter, pickle aroma, mousy and geranium.

6. Other Off Aromas
Very unpleasant odors associated with lactic spoilage include mousy and geranium-like aromas.

The mousy aroma has been attributed to the formation of a compound called acetyltetrahydropyridine. Two species of lactobacillus have been shown to produce these mousy odor compounds.

Sometimes a wine can develop a geranium-like odor which makes it undrinkable. This odor is caused by a compound known as 2-ethoxyhexa-3, 5-diene. This compound is produced from the decomposition of sorbic acid by the LAB. In sweet wines sorbic acid is often added to prevent the growth of unwanted yeast (yeast growth can cause refermentation). When the sorbic acid is attacked by LAB, 2-ethoxyhexa-3,5-diene is formed which imparts the geranium-like odor to the wine. To prevent this odor the growth of LAB in sweet wines containing sorbic acid should be controlled.

Factors Influencing the Growth of Lactic Acid Bacteria (LAB) in Wine
We have discussed the nature and extent of wine spoilage by LAB. In order to prevent wine spoilage by these micro-organisms, it is important to know the various factors that influence the growth of these microbes in wine so that one can manipulate these factors to reduce the risk of spoilage. The three important factors that deserve some consideration include:

1. Must/wine composition
2. Vinification practices
3. Interrelationships with other organisms

Must/Wine Composition
Wine pH - Wine pH is one of the most important factors influencing the growth of LAB. It affects the initiation and duration of malolactic fermentation MLF, it influences the type of species of bacteria that may develop in wine and it also affects the metabolic behavior of the organism and thereby determines the kind of by-products formed as a result of bacterial activity.
In the wine pH range of 3.0 to 4.0, the time needed for the completion of MLF decreases with an increase in pH. Bousbouras and Kunkee (1971) reported that at pH 3.15 it took 23.4 weeks to complete MLF; whereas at pH 3.83, it was completed in just two weeks.

Many researchers have noted the effect of pH on the species of bacteria that can grow in wine. Generally at pH below 3.5, the MLF is often dominated by Leuconostoc, whereas; above pH 3.5, species of Pediococcus and Lactobacillus seem to flourish. It should be noted here that many strains of Lactobacillus are involved in wine spoilage.

Another important pH effect not commonly realized is the effect of pH on the metabolic behavior of the organisms. For example at pH 3.5 and above, LAB are more likely to decompose sugars, tartaric acid and

citric acid. As mentioned earlier, fermentation of sugar leads to higher volatile acidity (VA) levels in wine. From the foregoing discussion it should be obvious that controlling wine pH is one of the keys to controlling wine spoilage by LAB.
Sulfur dioxide ($S0_2$) Sulfur dioxide is an effective germicide commonly used by the winemakers to control the growth of harmful bacteria. The SO_2 in wine exists in free and bound forms. All these forms remain in an equilibrium which is influenced by pH. Concentration of the molecular SO_2 form of free SO_2 which is also the most toxic form increases with a decrease in wine pH. Therefore, maintaining low pH is helpful in making SO_2 the most effective tool to control LAB. The bound form of SO_2 has also been reported to have a detrimental effect on LAB. In wine, SO_2 is bound to certain carbonyl compounds such as acetaldehyde. When LAB attacks the carbonyl compound, the bound SO_2 is released. It is this liberated free SO_2 that prevents further growth of the bacteria.

SO_2 is an effective germicide and concentrations of 0.8 ppm molecular SO_2 will be adequate to control the growth of LAB in wine.

Alcohol - Generally LAB can survive and grow in table wines. There is some variation between various species regarding alcohol tolerance. For example; Lactobacillus trichods has been found in wine containing 20% alcohol. The alcohol tolerance is influenced by pH and storage temperature.

Oxygen and Carbon Dioxide - Although microaerophilic conditions are desirable for the growth of LAB, the evidence suggests that a small amount of 02 may be necessary. It is however, widely recognized that the presence of CO_2 stimulates the growth of LAB. This may be a factor stimulating MLF in wines left on the lees which would contain a fair amount of dissolved CO_2. Kelly, Asmudson, and Hopcroft (1989) concluded that this was likely due to low levels of O2 as they observed the same effect using N_2.

Nutrients - LAB require a source of energy such as carbohydrates and inorganic salts. In addition they also need other growth factors such as vitamins and amino acids. Yeast autolysis (which occur during prolonged lees contact) resulting in increased nutrient content can render a young wine prone to attack by LAB.

Vinification Practices
Many vinification practices can influence growth of LAB in a winery. Some of the important practices include: fruit condition, must treatment (adjustment), clarification, fermentation conditions, skin contact time (in case of red wine), lees contact, wine clarification, storage and winery sanitation.

Sound fruit has a low population of LAB on the surface, therefore; using clean and healthy fruit is important in reducing the number of microbes that would enter the winery at harvest. Sulfur dioxide is often added at the crush. It is one of the most effective measures in controlling the growth of LAB. Winemakers not sulfiting the must at crush in order to reduce sulfite's in wine are taking a bigger risk in exposing their wines to bacterial spoilage. High pH musts usually contain low acid levels. The acidity and pH of such a must should be adjusted with tartaric acid additions before fermentation. This will enable fermentation to occur at low pH, and thus reduce the chances of spoilage by LAB. Clarifying white must by settling or other means reduces the suspended solids in the must. This practice is suggested for discouraging MLF in white wine.

Fermentation conditions affect the growth of LAB. For example, in case of a stuck fermentation, LAB can attack sugar and increase V A levels in wine. Controlling the fermentation so that it proceeds rapidly, evenly, and reaches dryness, is a sound enological practice to prevent any damage from LAB. A young wine left on the lees for a long time will be prone to MLF. This is due to the availability of nutrients released by yeast autolysis and a reduced CO_2 environment. For controlling LAB, early racking is recommended. Wine clarification, especially using tight filter pads or a .45 micron membrane filter will reduce the bacterial population and consequently the chance of spoilage.

Of all the winery practices, cleaning and sanitization of equipment and containers is one of the most important practices that a winemaker must employ to control the wine spoilage.

Interrelationships with Other Organisms
LAB does not seem to grow well in must during alcoholic fermentation. It seems that yeast has an inhibitory effect on the growth of LAB. This could be due to several reasons such as competition and depletion of nutrients by yeast, competition by natural yeast flora (eg. Pichia), formation of ethanol, SO_2 and other inhibitory compounds by the yeast.

Contrary to the antagonistic effects of the yeast, there are however, some reports that suggest that yeast may have stimulatory influences on the growth of LAB. For example, prolonged contact with the lees can

result in enrichment of young wine by yeast autolysis. This in turn can stimulate the growth of LAB.

Other microorganisms such as Botrytis cineria and acetic acid bacteria have been reported to have a stimulating effect on LAB. LAB are often found in association with acetic acid bacteria and there is some evidence indicating a symbiotic relationship between these organisms.

Bacteriophages are known to destroy the LAB. These phages have been isolated from wine. Not much is known about the inhibitory impact of these phages on LAB in wine and its influence on wine quality.

Recommendations to Winemakers

Since LAB are involved in MLF as well as wine spoilage, a winemaker needs to decide up front whether to encourage MLF.

If the choice is to encourage MLF (and avoid spoilage), then the following recommendations should be followed and MLF must be conducted under controlled conditions.

1. Use clean, healthy and high acid fruit.
2. Add a small dose of SO_2 at crush. (About 25-30 ppm based on must pH.)
3. Adjust the must pH if necessary. A pH range of 3.3 to 3.5 is desirable for MLF. Since MLF causes an increase in pH, it is advisable to conduct MLF at the lowest must pH as practically possible.
4. Inoculate the must with a pure starter culture of ML bacteria. The preferred time of inoculation is the 2nd or 3rd day after the alcoholic fermentation has begun. Low ethanol, low SO_2 and warm fermentation conditions favor MLF.
5. Take precautions to avoid a stuck fermentation. This would include not using overripe or moldy grapes, using a good dose of vigorously growing, pure culture of yeast, adding yeast nutrient and maintaining controlled temperature conditions. Do not allow fermentation temperature to exceed about 30° C or 86° F.
6. Monitor MLF and as soon as it is completed, treat the wine to prevent further growth of any LAB.

If the winemaker's choice is not to encourage MLF then the following recommendations should be followed as a guide to prevent MLF as well as spoilage due to LAB:

1. Use sound fruit for making wine.
2. Add SO_2 at crush, about 50 to 75 ppm based on must pH.
3. In a low acid and high pH must, add tartaric acid to bring the pH to 3.3 or lower.
4. In the case of white wine, clarify the must (reduce suspended solids) before fermentation.
5. Control fermentation temperature. Use well prepared, pure culture yeast starter. Use yeast nutrient if needed.
6. In the case of red wine, prevent must temperature from exceeding 85° F. Punching the cap and keeping the cap moist is important.
7. After the must is fermented dry, promptly rack the wine off the lees and add enough SO_2 to attain 0.8 ppm molecular SO_2 level.
8. Clarify and stabilize the wine and store in clean containers.
9. Clean and sanitize equipment and containers before processing the wine.
10. Sterile filter and store wine at cool cellar temperatures.

SUMMARY

Lactic acid bacteria are present on grapes, contaminated winery equipment and storage vessels. Some of the LAB primarily decompose malic acid and under certain conditions, attack sugar and malic acid. These are often involved in MLF and rarely in wine spoilage. Certain other LAB grows in low acid conditions; metabolize sugars (pentose), tartaric acid and glycerol. These are more dangerous organisms and cause serious spoilage. Conditions such as moldy fruit, low alcohol, low SO_2, high pH (3.5.and above), low acidity, presence of fermentable sugars and warm temperatures such as 25° C (78° F), favor the growth of LAB and can cause wine spoilage. Maintaining an adequate SO_2 level, low pH, and sanitary conditions during processing can prevent the spoilage.

Literature cited.

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