D-Lactic Acid Tests for Bacteria Contamination

By Mike Miller

Lactic acid bacteria (LAB) are important in wine production. They are responsible for the malolactic fermentation that occurs in many wines, reducing the wine's total titratable acidity, softening the wine,[1] producing a broader palate of flavors, and enhancing the wine's microbiological stability.[2] The lactic acid bacteria found in wine fall into four genera: *Lactobacillus, Leuconostoc, Pediococcus,* and *Oenococcus*, which is most frequently responsible for malolactic fermentation. Conventionally, they are further identified by their metabolic preferences:

general class	genus	basic carbohydrate	major metabolic product(s)
heterofermenters	*Lactobacillus* *Leuconostoc* *Oenococcus*	glucose	lactic acid + acetic acid + ethanol + carbon dioxide
homofermenters	*Pediococcus*	glucose	lactic acid (> 85%)
facultative heterofermenters	*P. pentosaceus,* *L. plantarum*, etc.	glucose (*or*) • pentoses (e.g., arabinose) •	lactic acid lactic acid + acetic acid

Of these wine LAB, all *Leuconostoc sp.*, all *Lactobacillus sp.* except *L. casei* (usually found in milk products), and all *Pediococcus sp.* at wine pH levels produce D-lactic acid from sugars.[18]

Unfortunately, these same bacteria may inhibit primary alcoholic fermentation,[9] and can also cause development of a range of off flavors known as lactic taint, *piqûre lactique* in France, and *spunto lattico* in Italy.

How does this occur? It's related to bacterial metabolic pathways. Under preferred conditions of moderate wine pH, sufficient nutrients, warmer temperatures, and low carbohydrates (sugars), lactic acid bacteria (LAB) convert L-malic acid to L-lactic acid, and deliver the improvements listed above. However, under many other conditions, such as stuck or sluggish fermentation, the malolactic LAB and/or contaminating LAB convert some of the remaining sugars into D-lactic acid and acetic acid, one of the major "incidents" in winemaking.[22] They can generate acrolein from glycerol which in turn reacts with anthocyanins to give wine a bitter taste.[20] They cause "ropy" wines by converting residual sugars into long chain polymers that result in an abnormal and unacceptable increase in viscosity. They produce excess levels of buttery diacetyl, cause "mousy" wines, and produce ethyl carbamate from arginine. They can form biogenic amines like histamine, tyramine, and putrescine, chemicals which cause allergic reactions in susceptible individuals.[3, 13, 14, 22] Under the right (wrong?) conditions LAB can metabolize the preservative sorbic acid, and give wine the aroma of geraniums.[19]

Where do LAB come from? Many strains of LAB have been found at low levels on grapes. Where fruit damage has occurred, levels are considerably higher. In one study, LAB were found on 9 of 21 batches of undamaged grapes, but on 16 of 22 batches with damaged grapes.[4] In another study, when sampled during fermentation, 31% of Oregon wines were found to contain detectable levels of Lactobacillus sp., with one winery showing a contamination rate of almost 80%.[10] LAB can also be introduced from inadequately sanitized pumps, valves, and transfer lines, as well as from almost-impossible-to-sterilize cooperage.[3] Given the conditions at crush of high sugars, warmer temperatures, and higher than desired pH levels, LAB can multiply rapidly. They generally decrease during primary fermentation as a result of their susceptibility to higher alcohol levels, but LAB populations can reemerge at troublesome levels during malolactic fermentation, or during aging and storage, if protections are inadequate.[11, 12, 23] Monitor often! Grapes themselves do not produce lactic acid. D-lactic acid,[3, 13, 14, 21] at levels above 300 mg/L,[13] is related to contamination!

How do we control them? Most LAB generally do not grow in lower pH wines; they exhibit reduced growth in wines with a pH below 3.5, and essentially no growth when the pH is below 3.2, a corrective action available with white wines but typically not reds.[2, 7] Higher alcohol levels (>13%) slow their growth, but are not lethal.[11] Many LAB are susceptible to SO2, especially at total SO2 levels above 70 ppm; however, since the *Oenococcus oeni* sp. usually preferred for malolactic fermentation are more susceptible to SO2 than *Pediococcus* sp. and *Lactobacilli*, alternate methods of control should be considered if MLF is planned.[16] Lysozyme has been found useful for control of LAB at levels of 100 - 250 mg/L, especially during crush, cold soak, and primary alcoholic fermentation when malolactic fermentation is planned.[5, 6, 8, 15] Contaminating LAB are also fairly susceptible to temperature. Control the temperature during malolactic fermentation at 20 - 23•C, and at 15 - 18•C afterwards.[13, 17]

Since grapes do not produce lactic acid, regular monitoring throughout winemaking and aging, i.e., regular measurement of D-lactic acid levels, can be used as an indicator of the onset of bacterial contamination and allows for minimally invasive yet effective control of lactic acid bacteria infections. The uncontrolled bacterial activity of lactic acid bacteria in wines can cause a drastic alteration of the quality of the final product**!** [21]

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