Spoilage due to lactic acid bacteria

Lactic acid bacteria (LAB) are the microorganisms that conduct malolactic fermentation (MLF) in winemaking, but they can also cause wine spoilage. Some of the more common questions about LAB spoilage are discussed below.

WHICH LAB ARE ASSOCIATED WITH WINE?

THERE ARE THREE main genera of LAB connected with grape must and wine – *Lactobacillus*, *Oenococcus* and *Pediococcus* – all of which can cause spoilage in wine. *Oenococcus oeni* is the species most commonly used for MLF.

WHAT DO THE DIFFERENT LAB LOOK LIKE UNDER THE MICROSCOPE?

The cells of *Oenococcus oeni* are spherical in shape with diameter approximately 0.5 –1.0 µm. They tend to exist as pairs or in long chains resembling a 'string of pearls'. *Lactobacillus* sp. typically appear as rod-shaped cells, approximately 0.5–1.0 µm x 1.0–2.5 µm. *Lactobacillus* are generally found in pairs of cells, but can also form chains of cells. Like *Oenococcus*, *Pediococcus* sp. cells appear spherical; however, they are larger than *Oenococcus* (generally about 1.0–1.5 µm in diameter). *Pediococcus* can appear as single cells, pairs of cells or as four cells in a clump. When four cells are together, each cell occupies a corner of a tetrahedron and the *Pediococcus* cells are said to be present as 'tetrads'. When observed, this morphology makes *Pediococcus* easy to recognise.

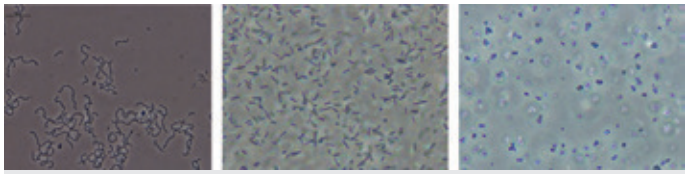

Figure 1 (L to R) *Oenococcus* sp., *Lactobacillus* sp. and *Pediococcus* sp. bacteria at 1000x magnification

WHAT ARE THE FAULTS LAB CAN CAUSE?

The main wine faults caused by LAB are summarised in the table on this page. While the AWRI has investigated cases of all of these faults, the first three (acidification, mousiness and geranium character) are the most common.

WHERE DO THE LAB COME FROM?

Low numbers (<10^3 colony forming units/g) of LAB are generally found on sound fruit and end up in must during the early stages of processing. However, high populations may develop on unsound fruit, for example, fruit that has split due to dehydration or bird damage. Once the fruit is damaged, LAB present on the grapes can multiply due to increased access to nutrients. Mechanical harvesting can also damage fruit and stimulate the growth of indigenous LAB during transport to the winery, especially when large distances are covered during hot weather. Populations of LAB can also develop in the winery and are often isolated from barrels and equipment that have not been properly sanitised, such as pumps, valves and transfer lines.

Fault	Description	LAB associated with the fault
Acidification	Production of high levels of acetic acid and lactic acid by fermentation of sugars.	*Lactobacillus*, *Pediococcus*, *Oenococcus*
Geranium character	Added sorbic acid is reduced to sorbyl alcohol by LAB, which rearranges in wine and reacts with ethanol to form 2-ethoxyhexa-3,5-diene, which imparts a geranium character.	*Oenococcus*, possibly *Lactobacillus*
Mousiness	Metabolism of amino acids, notably lysine and ornithine, can lead to formation of nitrogen-heterocyclic 'mousy' off-flavour compounds. These compounds impart a character reminiscent of a mouse cage.	Mainly *Oenococcus* and *Lactobacillus*
Acrolein/ bitterness	Metabolism of glycerol results in the formation of acrolein, which reacts with red wine phenolics to form a complex that imparts a bitter character.	*Lactobacillus*, *Pediococcus*
Mannitol off-flavour	Mannitol can be formed from reduction of fructose. Elevated levels of acetic acid and lactic acid are also present and the wine exhibits a vinegary-estery, slightly sweet taste.	Mainly *Lactobacillus*
Ropiness	Metabolism of glucose to form dextrin polysaccharide, which gives wine a viscous, oily character referred to as 'ropy'.	*Pediococcus*
Overproduction of diacetyl	Metabolism of citric acid or sugar to form diacetyl, which imparts a buttery or whey-like flavour.	Mainly *Lactobacillus* and *Pediococcus* (lower levels from *Oenococcus*)

HOW DO I AVOID SPOILAGE DUE TO LAB?

Spoilage can generally be avoided by a combination of sanitation, pH adjustment, use of sulfur dioxide (SO_2) with reference to the pH, minimisation of residual sugar and temperature control.

At the time of harvest, sanitation of grape bins between loads helps to minimise the build up of unwanted LAB populations and other microbes. Similarly, winery equipment, such as receival bins, crushers, presses, must pumps and lines, should also be regularly cleaned and sanitised to minimise microbial build-up.

The growth of *Lactobacillus* sp. and *Pediococcus* sp. is encouraged at higher (>3.5) pH. Consequently, once must tanks are mixed and the acidity parameters are known, tartaric acid can be added to adjust the pH to <3.5.

LAB are more sensitive to SO_2 than yeast and a molecular SO_2 concentration of 0.8 mg/L will inhibit their growth. It should be

noted, however, that the amount of SO_2 present in the molecular form depends on wine pH, so the SO_2 concentration should be adjusted based on knowledge of the pH.

Optimal yeast preparation and the use of fermentation management strategies to avoid stuck fermentations will minimise the concentration of residual sugar after fermentation, a substrate for LAB growth. Once MLF is complete, a large (40–50 mg/L) addition of SO_2 will help to kill off any residual LAB bacteria. Storage of wine below 18°C will also inhibit growth.

Finally, if viable LAB are detected during microbiological analysis of an at-risk wine (e.g. one with residual sugar or sorbic acid) before bottling, sterile filtration through 0.45µm membranes may be required to completely remove the bacteria and achieve microbial stability.

For more information about LAB spoilage, please contact the AWRI helpdesk on helpdesk@awri.com.au or 08 8313 6600.

FURTHER READING

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