Winemakers are practitioners of microbiology; from the time that grapes are picked and crushed, through fermentation, maturation and ageing, winemaking is a microbiological process, shaped by a community of bacteria and yeast. Micro-organisms, particularly bacteria, are the most numerous and ancient forms of life, and have successfully colonised every possible ecological niche on the planet (see breakout box 1 for more information on bacteria), including the myriad of potential habitats in a vineyard and winery, bacteria and yeast that colonise grape must and wine typically originate from vines, grapes, winery processing equipment, surfaces in the winery, and, perhaps, even the winemaker. This ‘natural microflora’ includes several dozen species, with bacterial representatives largely coming from the lactic acid bacteria (LAB) and acetic acid bacteria (AAB). Of the yeasts, there are many species but once fermentation begins and alcohol levels start to rise, *Saccharomyces cerevisiae* predominates.

This article will focus on the bacterial component of the microflora that is associated with winemaking, considering first the ‘good guys’ then the winemakers’ foes.

**BACTERIA – THE WINemaker’S FRIEND**

The role of bacteria in winemaking is well established. Nearly 150 years ago, the father of microbiology, Louis Pasteur, noted the presence of bacteria in fermenting grape juice, and their capacity to produce lactic acid. Some 30 years later, in the 1890s, Müller-Thurgau showed that bacteria, not yeast, were required to reduce acidity in wine, by a process we now know as malolactic fermentation (MLF). The importance of MLF became evident in the production of Burgundy and wines from California, where it was realised that MLF improved wine quality; practices were subsequently employed to ensure of its occurrence.

It was not until the late 1960s that the principal bacterium responsible for MLF was characterised. At that time it was identified as *Leuconostoc oenos*, later to be reclassified as *Oenococcus oeni*. Over the next 40 years, *O. oeni*’s role in winemaking was studied intensely. What we have learnt from this research is that deacidification of wine is not the only benefit of MLF; this process contributes in several other ways to wine quality. For example, the metabolism of malic acid is the removal of tartaric acid, which is a mineral constituent of grapes, and the acidification process provides a natural means of controlling the malic acid content of wine.

Some interesting facts about bacteria

- bacteria occupy every ecological niche on earth: fresh water, salt water, soil, acidic hot springs, deep in the Earth’s crust, on and in organic matter, including plant and animal bodies
- bacteria are abundant: there are typically 40,000,000 bacterial cells in a gram of soil, 1,000,000 in a millilitre of fresh water; they form much of the world’s biomass (there is an estimated five nonillion [5×1030] bacteria on Earth)
- it is estimated that approximately 1% of all bacteria have been described
- bacteria are small, so small that you cannot see them without the assistance of a microscope at 1000× magnification. Most are about 0.5 - 5.0 micrometers (μm) in length (a micrometre is one thousandth of a millimetre). One exception is Thiomargarita namibiensis, a bacterium found in ocean sediments off the continental shelf of Namibia, which is 0.1-0.5 mm in length, and can be seen with the naked eye
- there are approximately 10 times as many bacterial cells as human cells in the human body (a large proportion are found on the skin and in the digestive tract)
- without bacteria we would not be able to ingest some of the nutrients in our food
- bacteria are economically important and are used in numerous industrial processes: waste processing, bioremediation, oil spill clean-ups, biological pest control, and production of therapeutic proteins
- bacteria are used in fermentation processes to produce a large array of foods, including yoghurt, cheese, pickles, sauerkraut, soy sauce, cocoa, coffee, vinegar and wine.
of a potential carbon source for spoilage bacteria, thereby imparting microbial stability to wine. In addition, the production of secondary metabolites by \textit{O. oeni} augments the sensory qualities of wine; in other words, MLF imparts aroma and flavour attributes to wine.

Fermentation-derived compounds, originating from yeast and bacterial metabolism during fermentation of grape must, include many different types of volatile chemicals. Some of these compounds are found in wines at or above their sensory threshold. These compounds contribute flavours and aromas described as buttery, fruity, berry, floral to soap, fusel, solvent to vinegar, rancid and cheesy. Data on the effects of MLF on volatile fermentation-derived compounds in wines (from numerous different red and white wine trials) show that there is a consistent increase in most of the ethyl esters and a decrease in acetate-esters. The concentration of fermentation-derived compounds is also affected by the bacterial strain chosen and the timing of MLF inoculation during winemaking (Figure 1). For example, the level of buttery character can be managed relatively easily during MLF with the selection of an appropriate \textit{O. oeni} strain and winemaking practices.

One important group of flavour compounds present in the grape in a sensorially inactive form is the aglycones. These compounds are grouped broadly according to their chemical structure: norisoprenoids (e.g. damascenone), volatile phenols and other benzene derivatives (e.g. raspberry ketone), monoterpenes (e.g. linalool, nerol and geraniol) and aliphatics (e.g. hexanol). Prior to fermentation, volatile aglycones are bound to sugar groups, rendering them inactive (i.e. non-volatile), in this form they are known as glycosides. It has been demonstrated that \textit{O. oeni} and other LAB have glycosidase enzymes which release a range of aglycones from the sugars they are anchored to, thereby releasing aroma compounds, often to concentrations above their sensory threshold, and consequently positively influencing the wine aroma.

Another important source of wine aroma and flavour originates from the oak barrel: oak-lactones, for example, impart vanilla and coconut characters to the wine. Several non-volatile oak-lactone glycosidic precursors have recently been identified by researchers at the AWRI. The ability of wine associated yeast and bacteria to release oak-lactone was tested. Yeast (\textit{Saccharomyces, Hanseniaspora} and \textit{Dekkera/Brettanomyces} species) exhibited minimal ability to cleave the sugar from the precursor, however, \textit{O. oeni} was able to efficiently liberate oak-lactone: up to 80% was liberated by this bacterium compared to less than 20% by \textit{Lactobacillus} species. This supports anecdotal evidence in the wine sector that MLF contributes to enhancing the oak character in wine.

**BACTERIAL FOES**

Clearly, wine bacteria play an important and positive role in stabilising wine and shaping its sensory profile. However, there are also some real troublemaking bacteria that the winemaker has to battle. Unwanted bacteria can appear at any stage during winemaking and can cause a range of spoilage scenarios that winemakers are taught about in their oenology studies: mousy taint, bitterness, geranium notes, volatile acidity, oily and slimy-texture (ropy wines), and overt buttery characters (Figure 2).

What constitutes bacterial spoilage? Bacteria are winemakers’ foes when, for example, they produce metabolites with undesirable aroma descriptors, at concentrations above sensory thresholds. This, however, is complicated by the fact that the desirability of many compounds in wine is concentration-dependent. For example, the buttery or butterscotch aromas of diacetyl (an intermediate of citric acid metabolism in LAB) are generally regarded as pleasant and add complexity to wine at concentrations below 4mg/L. However, above this, diacetyl may become objectionable with overt buttery notes.

![Figure 1](https://via.placeholder.com/150)

Figure 1. Fatty ethyl esters that contribute to fruity and berry aromas in wine include ethyl 2-methylbutionate, ethyl 3-methylbutinate and ethyl 2-methylpropanoate. This figure shows: A) Changes in ester concentrations following MLF in Cabernet Savignon inoculated with four different \textit{O. oeni} strains after alcoholic fermentation (sequential MLF inoculation). B) Changes in ester concentrations in Shiraz wine using different MLF inoculation regimes with one \textit{O. oeni} strain (co-inoculation, inoculation at mid-alcoholic fermentation, inoculation at pressing or after alcoholic fermentation (sequential)).

![Figure 2](https://via.placeholder.com/150)

Figure 2. Summary of wine spoilage scenarios due to bacterial metabolism, including: diacetyl production, organic acid metabolism leading to a range of potential problematic outcomes, development of bitterness, geranium notes, mousy off-flavour, and ropy wine.
Even though the biochemistry and microbiology of most bacterial spoilage scenarios are well understood, and the winemaker understands how to control bacterial foes, spoilage still occurs from time to time presumably because the winemaker has not identified the problem early enough. In this context it is important to remember that perception of aromas and flavours varies from one individual to another: mousy off-flavour, perceived on the back palate as a persistent aftertaste reminiscent of caged mice, is, for example, not recognised by everyone. Hence, it is important when monitoring for spoilage during winemaking to ensure that tasting is done by more than one person.

Thus far described are the well-known bacterial spoilage scenarios. However, wine spoilages that have arisen in the last few years include the development of ‘plastic-like’ off-flavours, predominantly in wines produced from sub-optimal fermentation. Recently, we identified indole as being linked to ‘plastic-like’ off-flavour in wines. Even though yeast produce the highest concentrations of indole during alcoholic fermentation, wine LAB species, particularly Lactobacillus lindnerii were also able to produce indole.

Bacterial-induced wine spoilage can also occur in the bottle. Acetic acid bacteria (AAB) are part of the natural winemaking microflora, however, unless provided with conditions suitable for their growth they just ‘hang around’ not causing any problems. Give them oxygen and they flourish. Therefore, wine is at high risk of spoilage by AAB during prolonged barrel maturation. Poor management during bottling and subsequent storage of red wine can also give rise to spoilage.

One way of controlling growth of spoilage bacteria is with the addition of sulfur dioxide at various stages during vinification. Another strategy is maintaining low wine pH (high wine acidity), as was recognised in the 1940s by Australian scientists John Fornachon and Ray Beckwith. However, while these approaches are effective in controlling most troublesome bacteria, recalcitrant foes might require the use of emerging technologies that have been used successfully in other food and beverage industries. These include pulsed electric fields, ultra high pressure, ultrasound or UV irradiation, and natural products including bacteriocins and lysozyme (breakout box 2).

**SUMMARY**

Bacteria are essential to life on our planet and are critical in winemaking for that perfect glass of wine. For example, O. oeni metabolism during MLF deacidifies wine, imparts microbial stability and is able to alter the volatile composition and sensory profile of wine. Not all bacteria are friendly. Bacterial foes can spoil wine in many ways and therefore must be kept in check. As a practitioner of microbiology it is the winemaker’s job to fashion fermentation with friends and fight off the foes.

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**FURTHER READING**


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**Fending off bacterial foes**

**Traditional approaches**

- manage the pH of the grape must and wine (maintain pH 3.5)
- use SO2 at different stages during vinification to minimise growth of unwanted bacteria
- filtration of wine prior to bottling
- dimethyl dicarbonate (DMDC), usually to grape must

**Natural product additives**

- lysozyme – can be used at various stages throughout vinification
- bacteriocins

**Emerging technologies** (used successfully in other food and beverage industries)

- ultrahigh pressure – inactivates microorganisms without affecting flavour compounds
- high power ultrasound – high-energy microbubbles used in food processing
- UV irradiation – used in recirculated brines and killing fungi on grapes
- pulsed electric fields – a potential alternative to pasteurisation without loses of organoleptic qualities

* - not approved for use in Australia

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