Nº5 DIACETYL PRODUCTION BY WINE BACTERIA UNDER CONTROL

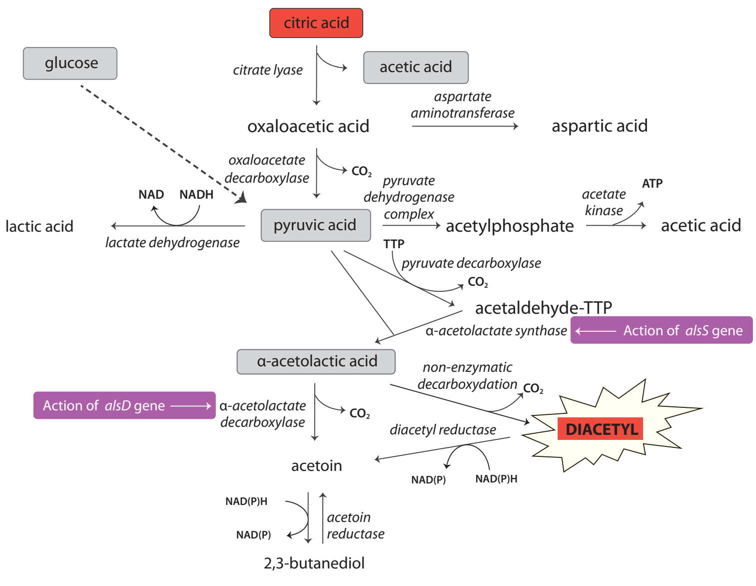
DIACETYL AND SENSORY IMPORTANCE IN WINE

Diacetyl is easily identifiable in wine because of its very low aroma threshold (0.2 mg/L in Chardonnay to 0.9 mg/L in Pinot Noir and 2 mg/L in Cabernet Sauvignon) and its distinctive buttery aroma. In some instances, diacetyl is a desirable attribute and in other cases it is undesirable. In low concentrations, it can be responsible for toasty and nutty aromas, while at high levels it imparts a buttery aroma. Formation of diacetyl increases when MLF proceeds because it is closely linked to the growth of wine bacteria and particularly their metabolism of citric acid. That's why the choice of wine bacteria plays a crucial role in the final concentration of diacetyl.

THE RELATIONSHIP BETWEEN CITRIC ACID, BACTERIA GENETICS AND DIACETYL

The metabolism of citric acid by *Oenococcus oeni* results in the production of flavor compounds, such as diacetyl, acetoin, and 2,3 butandiol (Fig. 1), and also helps to regulate intracellular pH (survival of bacteria) during the winemaking process. Each selected bacteria has its own properties and kinetics of citric acid consumption, which impact diacetyl production.

New research confirms that the bacteria strain is key because diacetyl formation is strongly regulated by the alsD (α-acetolactate decarboxylase) gene (Mink et al., 2014). The expression of this gene is *Oenococcus oeni* strain specific and it is directly linked with their metabolism of diacetyl production. If a strain of *Oenococcus oeni* has a very active alsD enzyme, it will produce less diacetyl and produce more butandiol, whereas, if alsD is less expressed - and in parallel alsS (α-acetolactate synthase) enzyme is more active, then more diacetyl will be produced by the bacteria .



SELECTED BACTERIA AND DIACETYL PRODUCTION RATE

This highlights that the concentration of diacetyl produced is not only related with the citric acid consumed but also with gene expression of *Oenococcus oeni*. That's why each bacteria strain has its own diacetyl production rate and some can degrade more citric acid for a lower production of diacetyl.

In our range, *O.oeni* **O-Mega™** has the lowest diacetyl formation power, despite its good capacity to degrade citric acid. Vincent Gerbaux (IFV 2018) studied **ALPHA™**, **O-Mega™**, and **Beta™** in Chardonnay and Pinot Noir wines. Table 1 shows the average values for citric acid consumption and diacetyl production where **O-MEGA™** was degrading more citric acid than **ALPHA™**, but is producing less diacetyl.

	Selected Wine bacteria	Citric acid consumption	Diacetyl production (µg/L)
Average values	ALPHA™	47%	619
	O-MEGA™	57%	225
	BETA™	70%	966

(Table 1)

Many studies and field trials led to the characterization of our selected wine bacteria for diacetyl production (Table 2)

	Diacetyl production **when used in sequential inoculation**	**Speed of citric acid degradation**
ML-PRIME™ / MT01	NO production	No degradation of citric acid
O-MEGA™ / VP41™	Very low to no production	Very low speed Degradation of citric acid after the end of MLF
ALPHA™ / L31™	Moderate production	Moderate speed Degradation of citric acid during MLF
PN4™ / BETA™ / MCBB	High production	Fast to very fast speed – Early degradation of citric acid during MLF

Table 2. Strain specific citric acid metabolism and diacetyl production by commercial wine lactic acid bacteria in sequential inoculation

TIMING OF INOCULATION AND DIACETYL MANAGEMENT

Some vinification factors (pH ,temperature, air contact, yeasts lees contact, SO_2, type of oak) can also impact the final concentration of diacetyl in wines, as well as the timing of bacteria inoculation.

During co-inoculation (inoculation of bacteria 24 H or 48H after yeast addition), diacetyl concentration in the wine peaks when there is still a high percentage of viable yeast is present. Under these reductive conditions, diacetyl can be reduced to 2,3 butandiol, which has a much higher perception threshold and doesn't contribute to the buttery flavor. **Thus, even if a wine bacteria is a high diacetyl producer, when used in co-inoculation, the final wine will have little buttery attributes and very low diacetyl concentration.** Contrary, sequential inoculation after alcoholic fermentation and a racking step results less reduction of diacetyl. **Thus sequential MLF can have higher diacetyl concentrations depending of the wine bacteria strain**.

SUMMARY

Choosing a suitable selected wine bacteria strain known as high diacetyl producer or as low diacetyl producer, with the proper timing of inoculation allow to drive the diacetyl content to help define the wine style.

	Favor diacetyl production	**Limit diacetyl production**
Selected wine bacteria	Sequential inoculation and select one of these wine bacteria: Lalvin MCBB™, BETA™, PN4™, ALPHA™	Co-inoculation. For all our wine bacteria For a sequential inoculation, select one of these wine bacteria: Lalvin MT01, VP41™, O-MEGA™, ML-Prime™