# ML01 strain

## Metabolic engineering of *Saccharomyces cerevisiae* in the winemaking industry

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## Introduction

The correlation between wine organoleptic properties, its chemical composition and fermentation conditions has been thoroughly studied for many years. Therefore, the control of the sensorial quality of wine through fermentation and composition regulation is one of the main goals of the R&D efforts in the winemaking industry

One proposed way of altering these would be the genetic and metabolic engineering of *Saccharomyces cerevisiae*, the most widely used yeast species in wine fermentation

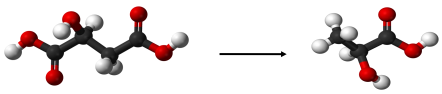
## Objectives and methodology

The goal of this bibliographic research project is to study the purposes, methodology and implications behind genetic engineering in the winemaking industry

To accomplish this, several examples were studied, such as the engineering of aromatic thiol compounds release to make flavour active wine yeasts, or the increase in esters and higher alcohols synthesis to further improve wine's taste and aroma

Out of all the examples, one case was selected as the most representative of this particular research area: the metabolic engineering of ML01, a commercial recombinant strain of *S. cerevisiae* used in the U.S winemaking industry

## Malolactic fermentation



**Malic acid** **Lactic acid**

Malolactic fermentation transforms malic acid, a secondary product of alcoholic fermentation, into lactic acid. The process is usually carried out by lactic acid bacteria that are added to the wine culture after the alcoholic fermentation is well over. Malic acid elimination decreases wine's acidity and ensures that it has a sweeter and more refined taste

## Proposal

Doing both alcoholic and malolactic fermentation simultaneously would reduce fermentation time, drastically improve the efficiency of winemaking processes and assure that the produced wine has an adequate taste

## Problem is…

Lactic acid bacteria do not carry out alcoholic fermentation with enough efficiency and *S. cerevisiae* cannot metabolize malic acid

## The solution

Modifying the genome of *S. cerevisiae* to make it capable of carrying out malolactic fermentation

## Metabolic engineering of ML01

1. Two genes necessary for malolactic activity were derived from lactic acid fermentative microorganisms used in winemaking processes

***mae1* gene**
**(malate transporter)**

*Schizosaccharomyces pombe* → *mae1*

*Oenococcus oeni* → *mleA*

***mleA* gene**
**(L-malate to L-lactate conversion)**

2. Through a series of clonings using yeast and bacterial vectors, the PGK1p glucose-induced promoter was added, along with its corresponding terminator and the URA3 flanking sequences

*URA3* *PGK1p* *PGK1t* *URA3*

URA3 is a *S. cerevisiae* gene involved in *de novo* pyrimidine nucleotide synthesis

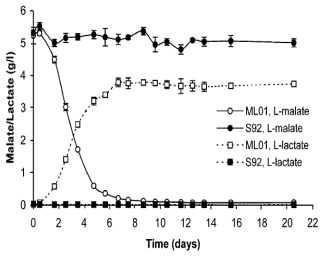
3. A malolactic integration cassette was constructed

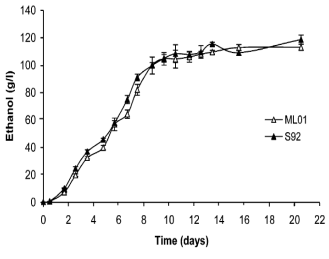
*URA3* *PGK1p* *mleA* *PGK1t* *PGK1p* *mae1* *PGK1t* *URA3*

4. The cassette was co-transformed in *S. cerevisiae* cells of the S92 *wildtype* strain

5. The malolactic cassette was successfully integrated within one *URA3* locus through homologous recombination and the ML01 recombinant strain was thus created

## Results




The modifications introduced in *S. cerevisiae* allowed the ML01 strain to carry out malolactic fermentation without losing any of its proficiency at alcoholic fermentation

ML01 was also subjected to a series of genomic analyses by the FDA. It was found that:

-The recombinant strain did not contain any bacteria-derived DNA or antibiotic resistances

-It had few mutations in the malolactic codifying sequences, even after a hundred generations in culture

**The ML01 strain is considered safe and stable, but only in the U.S.**

FDA Approved

## Conclusions

-Metabolic engineering has already taken its first steps towards its application in the wide winemaking industry

-Several legal restrictions still remain (specially in the European Union)

-The quality of wine and its production efficiency is expected to improve should modifications like these be safely implemented

## Bibliographic references

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