A QTL approach of the molecular basis of sulfite and sulfide production by wine yeasts

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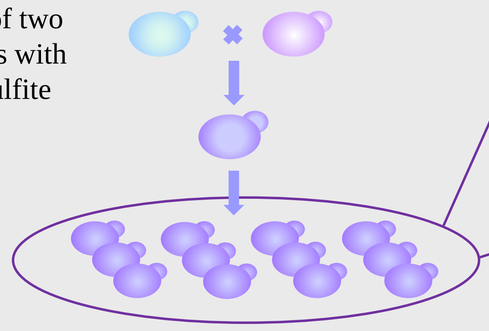
Enological yeasts contribute greatly to the final aromatic balance of wines through the production of volatile compounds of interest; nevertheless, they can also be responsible for the production of negative off-flavours, such as sulfur compounds. Sulfite and sulfide are two of those compounds whose production has to be controlled. The sulfate assimilation pathway has been widely studied, however, little is known about the molecular basis responsible for the differences in sulfite and/or sulfide production between yeasts strains. In this project, we implemented a QTL mapping approach to identify the genetic determinants of the low SO_2 production of a wine yeast strain.

QTL mapping strategy

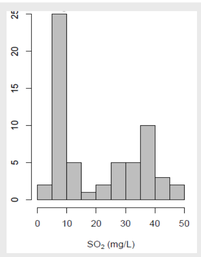
Selection of two wine yeasts with opposite sulfite production



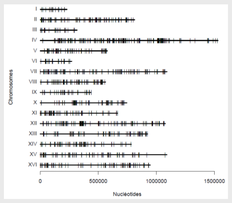
Constitution of a recombinant population

Phenotyping:
Enological conditions
Study of the phenotypes distribution through the population

Genotyping:
DNA microarray (Affymetrix)
Identification of molecular markers



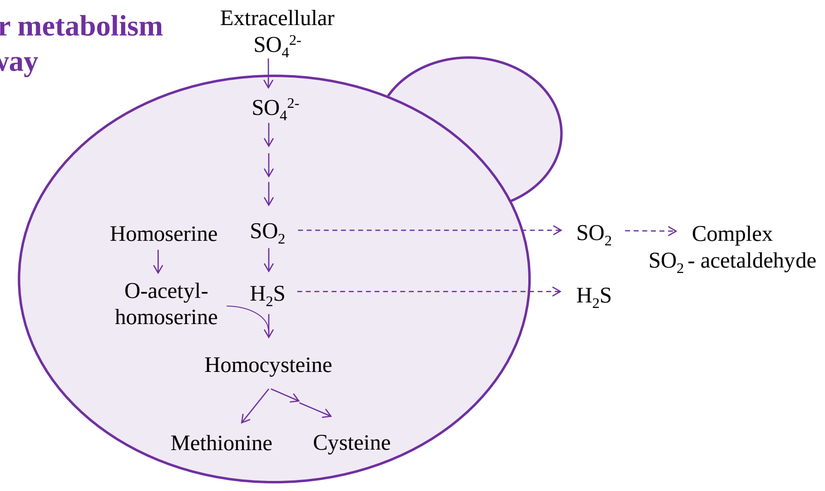
Distribution of the SO_2 production frequency



Physical map of molecular markers

Linkage analysis

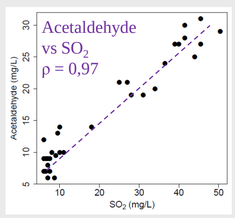
Sulfur metabolism pathway

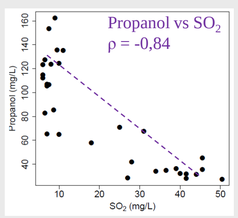


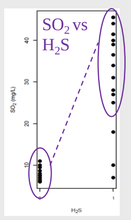
Identification and dissection of a QTL on the XIVth chromosome

Involvement of a double QTL region in 4 phenotypes of interest

A correlation analysis between the phenotypes of interest reveals strong relationships:



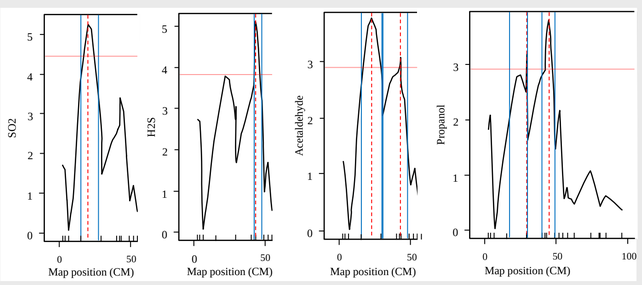




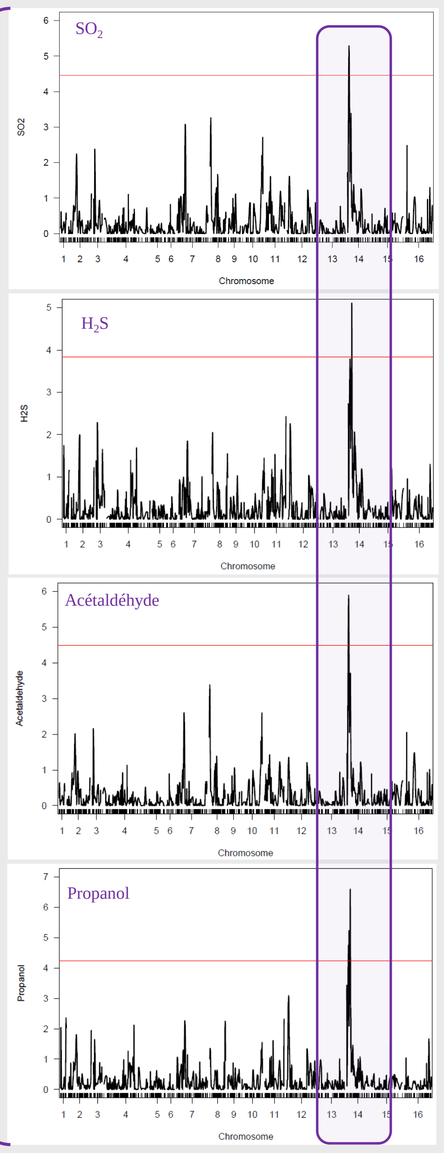
A linkage analysis reveals that a QTL region located on the XIVth chromosome is linked to four phenotypes of interest:

- SO_2 production
- H_2S production
- Acetaldehyde production
- Propanol

An accurate observation of the QTL region point out two peaks of LOD score:

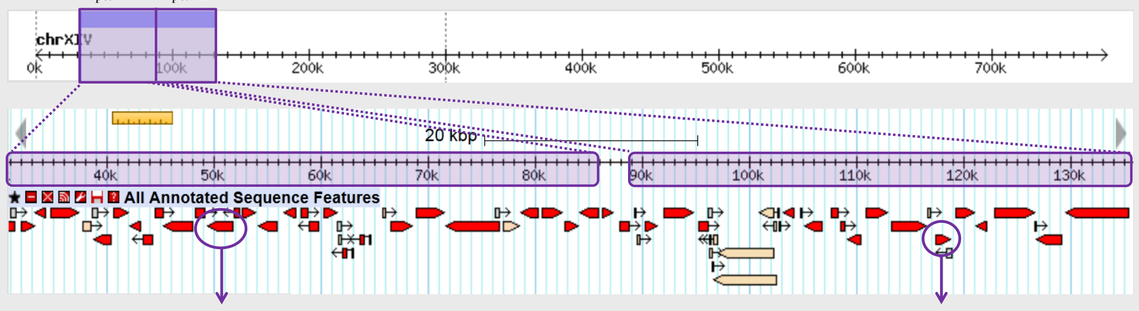


Zoom on the QTL region on the XIVth chromosome



LOD score curves (probability of the location of a QTL) versus position along the genome (5% significance threshold)

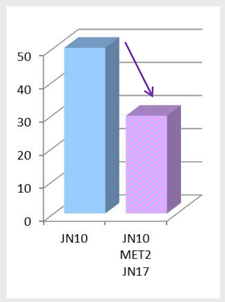
Identification of two candidate genes in the QTL region



SKP2 (YNL311C):	Gene	*MET2* (YNL277W):
F-box protein part of an SCF ubiquitin protease complex; involved in regulating protein levels of sulfur metabolism enzymes (including MET14p)	Function	L-homoserine-O-acetyltransferase, catalyzes the conversion of homoserine to O-acetyl homoserine
2 non-synonymous SNP	Sequence comparison between parental strains	1 non-synonymous SNP

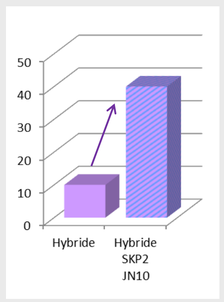
Functional validation and involved mechanism

Allelic replacements and hemizygotes construction



Allelic replacement of the *MET2* gene

The *MET2* allele of the JN17 (low sulfite producer strain) was introduced into JN10 (high sulfite producer strain)



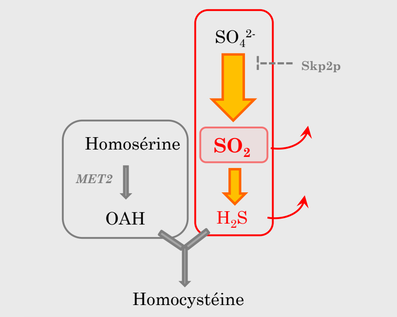
Hemizygote construction for the *SKP2* gene

The *SKP2* allele of the JN17 (low sulfite producer strain) was deleted into an hybrid between the JN10 and JN17 strains

Validation of the impact of the alleles of the low sulfite producer strain *MET2*^{JN17} and *SKP2*^{JN17} on the phenotype of SO_2 production

(The same validation has been performed on the other implicated phenotypes and gave similar results)

Schematic representation of the impact of the alleles on the sulfur metabolism

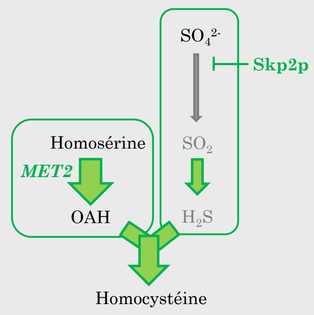


High sulfite producer strain

In a high sulfite producer strain:

- Low flux of homoserine conversion to O-acetylhomoserine
- High flux through the sulfate reductive sequence

Imbalance between the excess synthesis and the low incorporation of the sulfur intermediates SO_2 and H_2S leading to an higher liberation of those sulfur compounds



Low sulfite producer strain: JN17

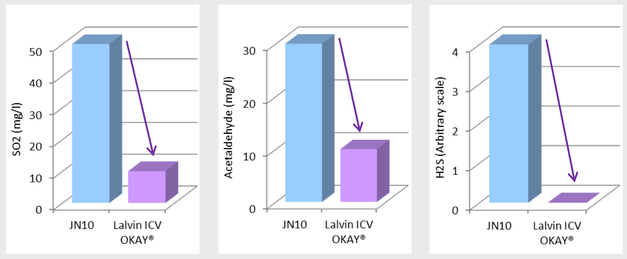
In the low sulfite producer strain JN17:

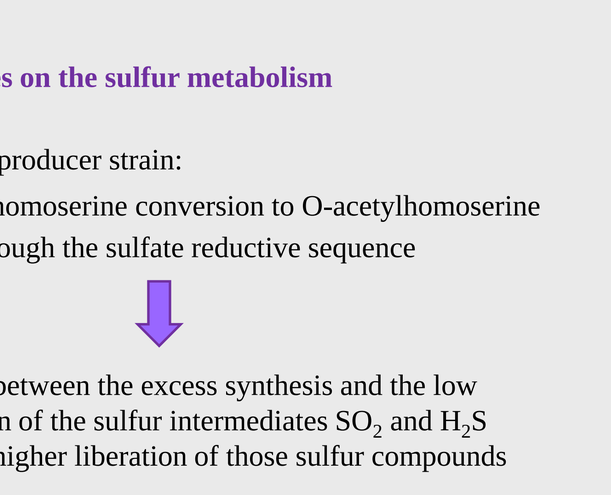
- High flux of homoserine conversion to O-acetylhomoserine
- Controlled flux through the sulfate reductive sequence

Combination of a lower synthesis of sulfur intermediates and a higher availability in carbon precursors leading to a very low SO_2 liberation and undetectable H_2S liberation

Construction of a new strain by backcrossing cycles

Transfer of both alleles through backcrossing cycles assisted by molecular markers to construct a new strain improved for its SO_2, H_2S and acetaldehyde production while retaining all the good properties of the parental strain





Thanks to a QTL approach, we succeed to decipher the mechanism underlying the low SO_2, but also low H_2S and acetaldehyde production of a wine yeast strain. We identify two genetically linked alleles responsible for a simultaneous control of the flux through the sulfate reductive sequence and of the conversion of O-acetylhomoserine to homoserine. We demonstrate that the transfer of those alleles in a high SO_2/H_2S/acetaldehyde producer strain is responsible for a huge diminution of their production (Patent*). Furthermore, we assume that the combination of those alleles is strong enough to control the production of those sulfur compounds in any other wine yeast strain and we succeed to transfer them into a good fermentative strain to improve it. This new strain, Lalvin-ICV OKAY®, has already demonstrated its very good enological properties in many trials (very low SO_2/H_2S/acetaldehyde production, very good fermentation activity, low nitrogen needs, intense fruity aroma, freshness and balance in mouth) and is now commercially available.

* Blondin, B., Noble, J., Sanchez I. *Méthode de contrôle de la production de sulfites, d'hydrogène sulfureux et d'acétaldéhyde par des levures.* PCT/IB2013/050623

5th Conference on Physiology of Yeast and Filamentous Fungi, June 4 - 7, 2013, Montpellier, FRANCE