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# RNAseq reveals different yeast behaviours in terms of nitrogen consumption and fermentative aroma production



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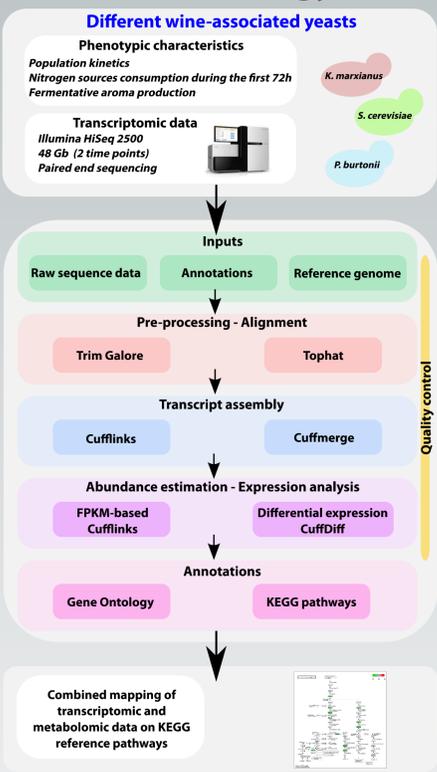
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Fermentative aromas play an important role in the organoleptic profile of wines. These compounds derive for a significant part from the break-down of nitrogen sources naturally found in grape juice, but their production also depends on the yeast strains driving the fermentation. During sequential inoculation of non-*Saccharomyces* yeasts with *Saccharomyces cerevisiae*, multiple interactions between yeasts may occur, including competition for nutrients that directly impacts fermentation performances and aroma production. Two unconventional non-*Saccharomyces* yeasts (*Pichia burtonii* and *Kluyveromyces marxianus*) were selected for displaying a behaviour different from that of *S. cerevisiae* in terms of nitrogen consumption and fermentative aroma production. The uptake of nitrogen sources was monitored together with fermentation and population kinetics. In parallel, total RNA was extracted at specific time points corresponding to lag phase (LP) and time where half of the maximal population (HMP) was reached. RNA sequencing was used to unravel these strain-specific behaviours at a molecular level.

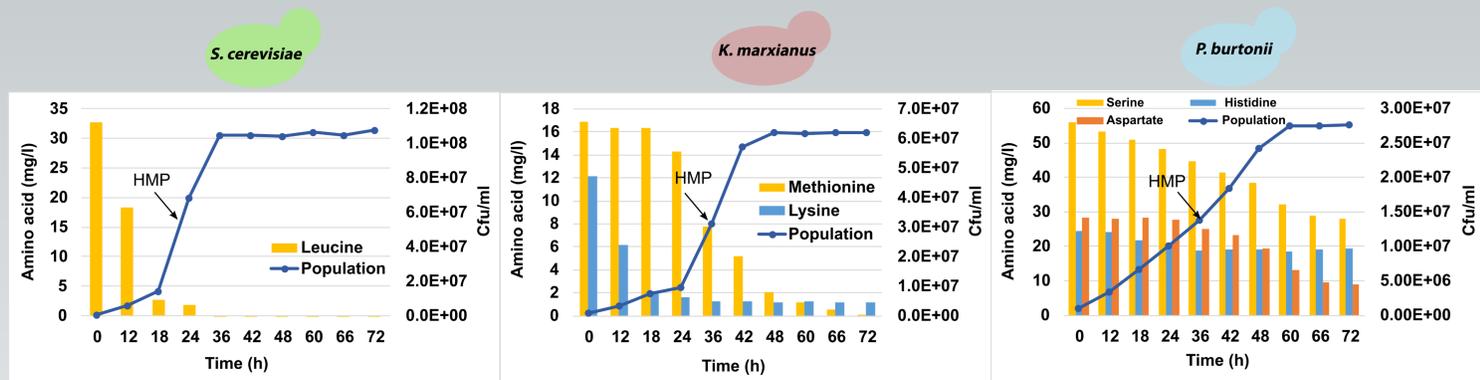
## Methodology



## Nitrogen consumption during the first 72 h of fermentation

Depending on the yeast strain and the amino acids considered, 3 behaviours were observed:

- ✓ Amino acids consumed continuously during the first 72 hours
- ✓ Amino acids consumed until the HMP was reached (but not depleted)
- ✓ Amino acids started to be consumed when the HMP was reached



All amino acids are consumed continuously and depleted within 36h (except GABA)

Arg, Asn, **Gln, Glu, Ile, Leu, Met, NH<sub>4</sub>, Phe, Ser, Thr, Trp, Val**

Ala, Asp, GABA, **Gly, His, Lys, Tyr**

Arg, GABA, Gln, Leu, Met, NH<sub>4</sub>, Ser, Thr, Trp

Gly, His, Ile, Lys, Val

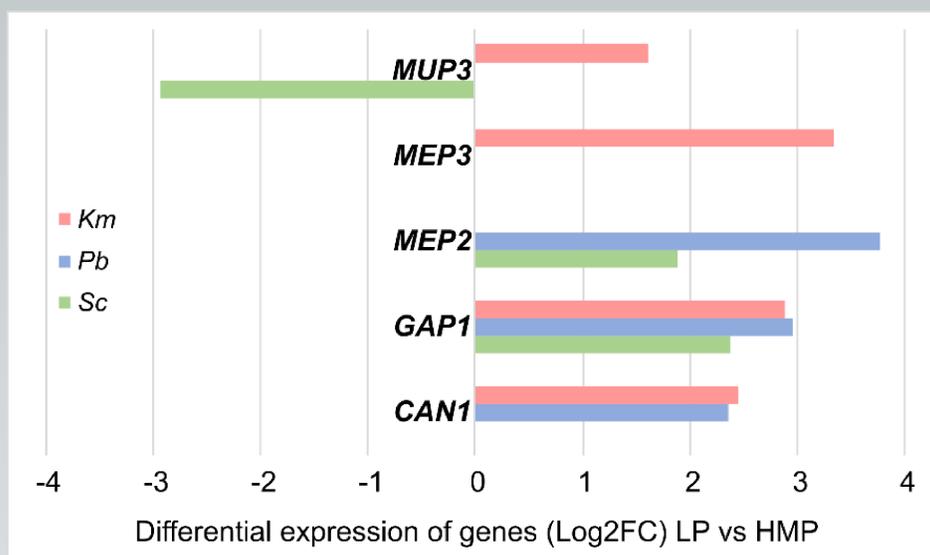
Ala, Asp, Asn, Glu, Phe, Tyr

In bold: Amino acids depleted within the first 72h

## Nitrogen source transporters

When HMP was reached:

- ✓ *S. cerevisiae*: almost all amino acids are depleted (except Arg, NH<sub>4</sub> and GABA)
- ✓ *K. marxianus*: 5 amino acids are still present in high concentration in the medium (Arg, Asp, Gly, His, NH<sub>4</sub>)
- ✓ *P. burtonii*: none of the amino acids is depleted



✓ GAP1 (general amino acid permease): up-regulated in the 3 strains

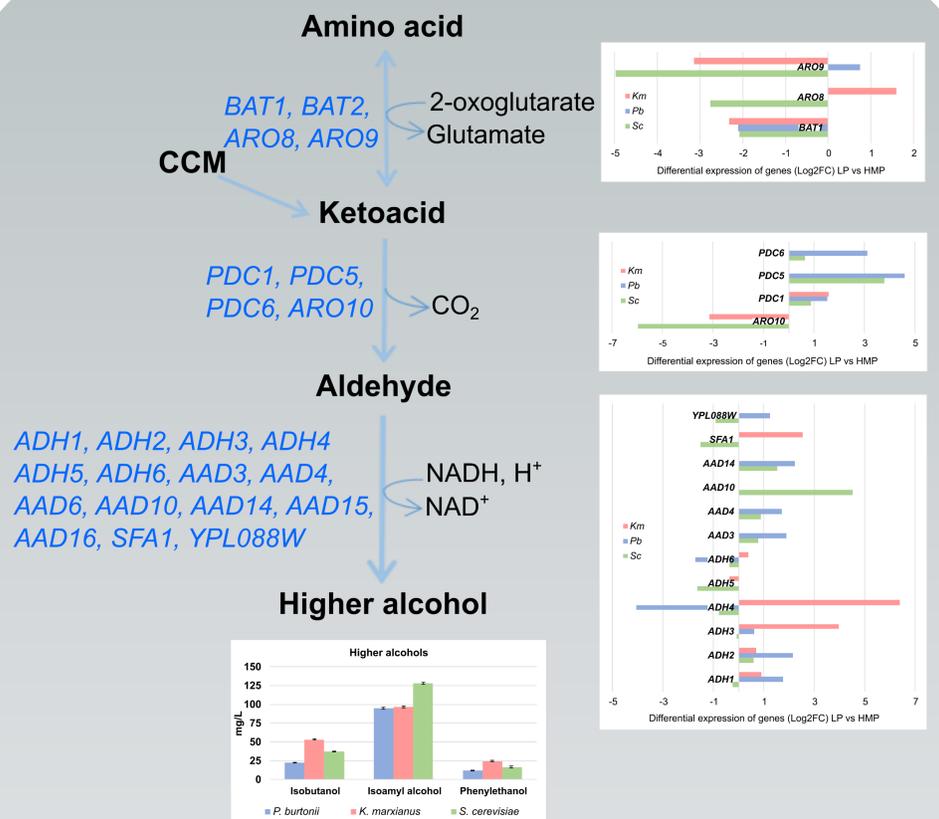
*K. marxianus* (compared to *S. cerevisiae*):

- ✓ Consumed 20% of NH<sub>4</sub> (60% for Sc) → Up-regulation of the low-affinity (**MEP3**) ammonium transporter; when Sc up-regulated MEP2 (high-affinity)
- ✓ Late uptake of Met → Up-regulation of Met transporter (**MUP3**), when down-regulated in Sc
- ✓ Late uptake of Arg → Up-regulation of **CAN1** (Arg permease)

Although *P. burtonii* overexpressed some nitrogen transporters, it:

- ✓ Was able to consume only 20% of YAN
- ✓ Struggled to ferment (only 25% of sugars consumed)
- *P. burtonii* not adapted to environmental conditions:
  - Poor adaptation to osmotic pressure?
  - Lack of oxygen?
  - Lack of nutrients (others than N)?

## Fermentative aroma production



*K. marxianus* (compared to *S. cerevisiae*):

- ✓ Up-regulation of ARO8
- ✓ Higher production of phenylethanol and isobutanol
- ✓ Same uptake of Phe, 60% of sugars consumed
- ✓ Up-regulation of ADH3, ADH4, SFA1 → Gene regulation different in *Km* (e.g. ARO8 vs ARO9) → Higher conversion of:
  - Phe into phenylpyruvate?
  - Aldehydes into higher alcohols?

*P. burtonii* (compared to *S. cerevisiae*):

- ✓ Up-regulation or higher expression of most of the genes involved in Ehrlich pathway
- ✓ Was able to consume only 20% of initial YAN and 25% of sugars → Ketoacid pool massively directed towards the higher alcohol production rather than the growth because of the low nitrogen consumption?

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