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Impact of nitrogen sources on the fermentative kinetic and aroma compounds production by non-Saccharomyces yeasts in alcoholic fermentation

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Over the past 50 years, the completion of wine fermentation and the formation of undesirable off-compounds have been mainly controlled through the use of selected starter strains of *S. cerevisiae*. Little attention was paid to the non-*Saccharomyces* yeasts, predominant in grape juice but rapidly outcompeted by *S. cerevisiae* during fermentation. However, the potential of some of these species, thank to their ability to produce fermentative aromas, to improve the sensory quality of wines is now recognized even if the metabolic specificities of non-*Saccharomyces* yeasts remain ill-characterised. To fill this shortcoming that is a hindrance to an efficient use of non-*Saccharomyces* strains during wine fermentation, the aim of this study was to investigate to impact of the nature of nitrogen source on the fermentation performances and the production of volatile molecules of 2 non-*Saccharomyces* species.

Material and Methods

Fermentation in synthetic media with **only one nitrogen source** (200 mgN/L) among 27 :

Ade, Ala, Arg, Asp, Asn, Cit, Cys, Cyt, GABA, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, NH₄, Orn, Phe, Pro, Ser, Thr, Trp, Tyr, Urea, Val

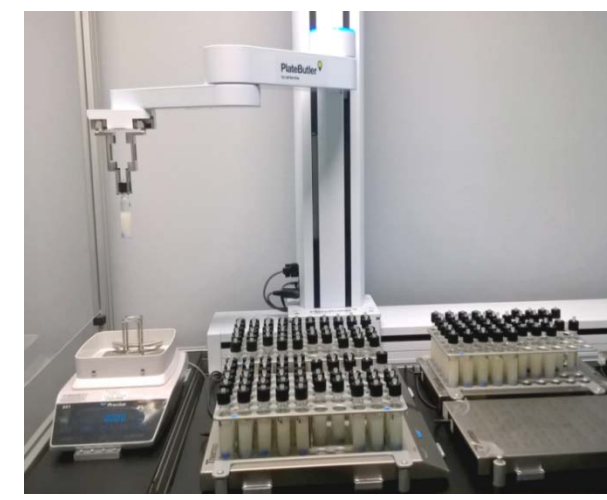
Study of 3 yeasts stains, naturally present on grapes :

- *Torulasporea delbrueckii* (TD 291)
- *Metschnikowia pulcherrima* (MP 346)
- *Saccharomyces cerevisiae* (SC 09)

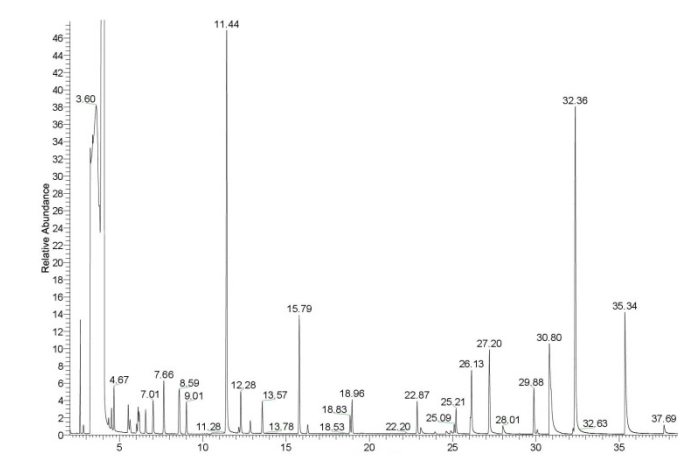
Fermentation in 15 mL-tubes – 1 AA per media

PhenoFerm : automatic weighting device

- Point each 4 h per tube to accurate determination of fermentation rate

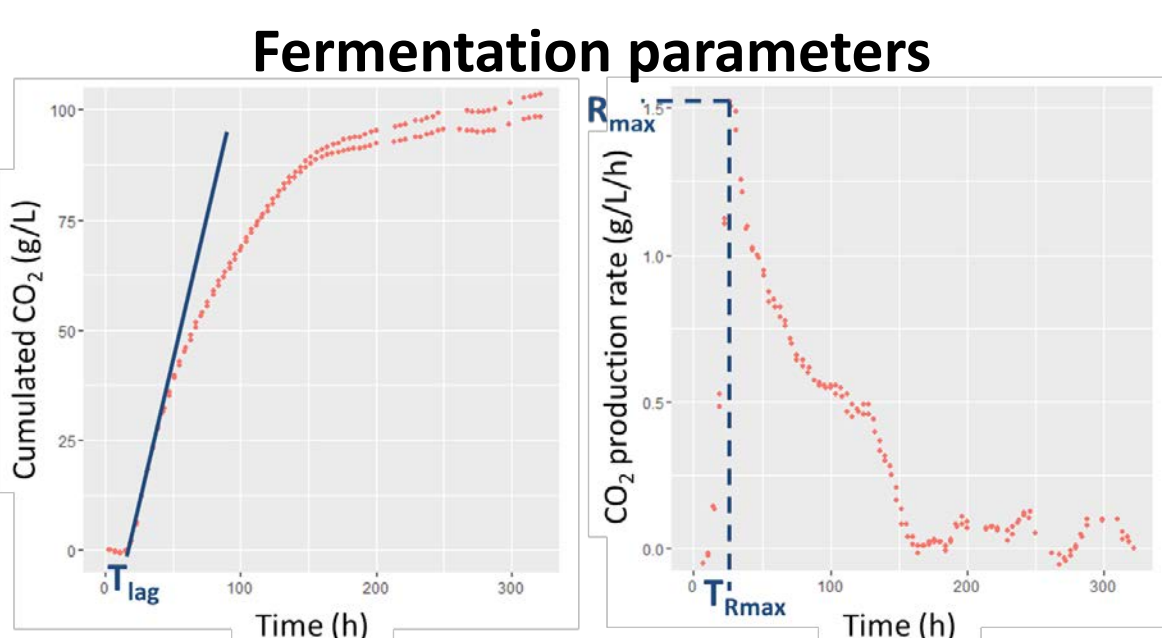


Offline determination of metabolites



Measure of concentrations of 35 aroma compounds at the end of fermentation by GCMS

Residual nitrogen at the end of fermentation determined by NOPA analysis (amino acids) or enzymatic kit (NH₄)



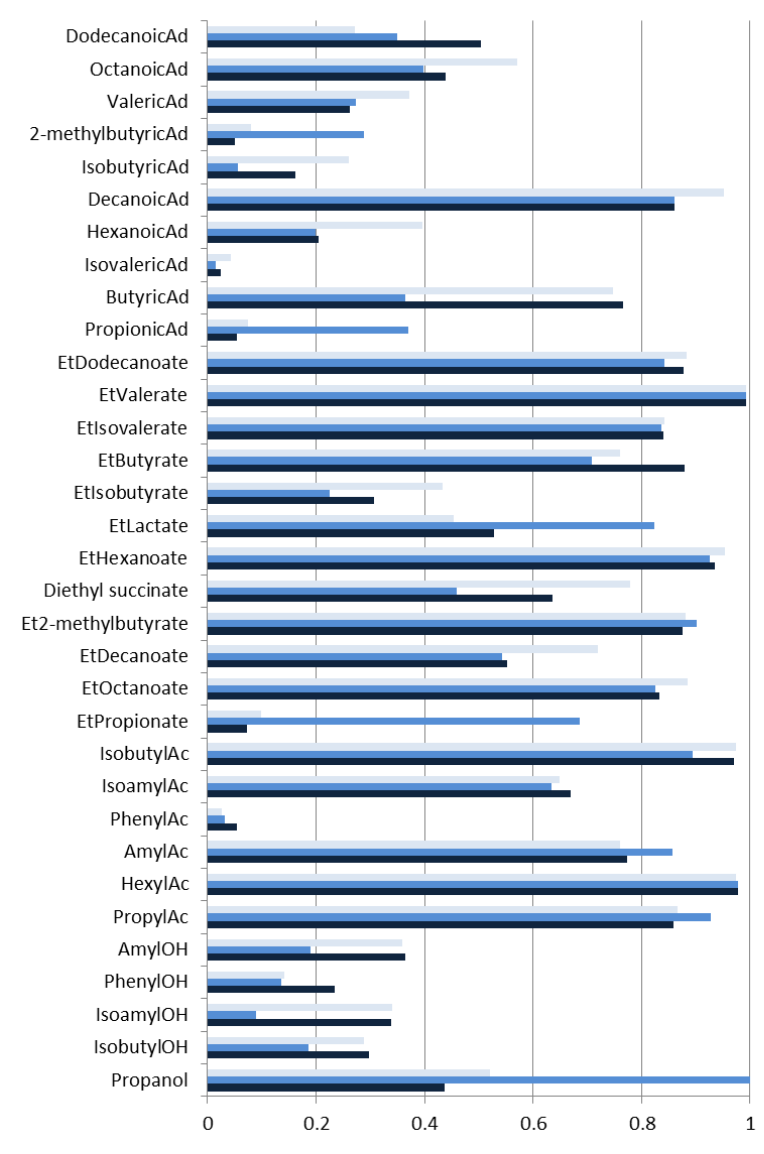
Determination of characteristic parameters :

- Tlag : lag phase (h)
- Rmax : maximal fermentation rate (g/L/h)
- Trmax : time to reach Rmax (h)

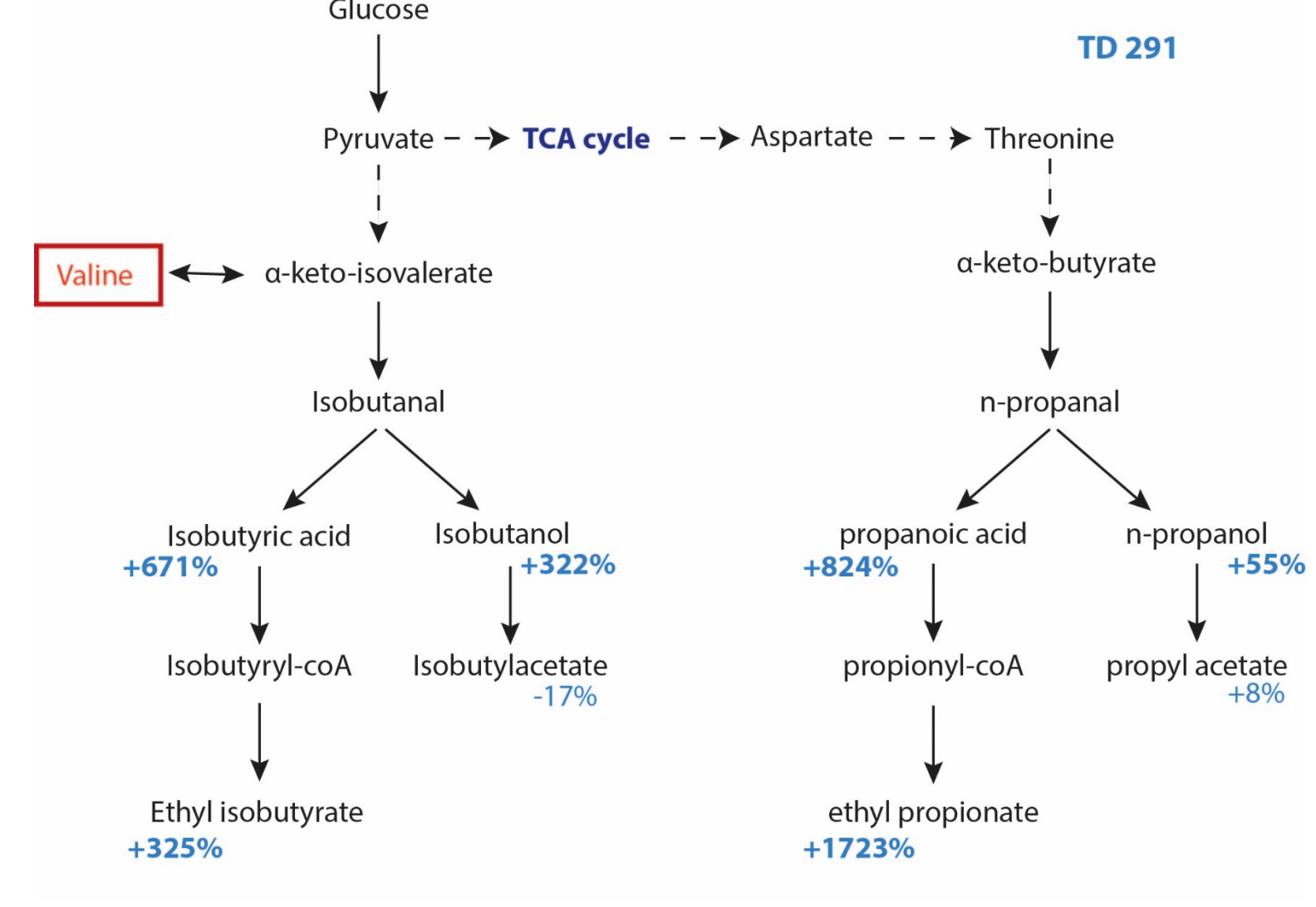
Production of aroma compounds

Production of volatile compounds during fermentation using a sole nitrogen source

Aromatic profiles of TD 291 in fermentation on sole nitrogen source (normalized concentrations) :



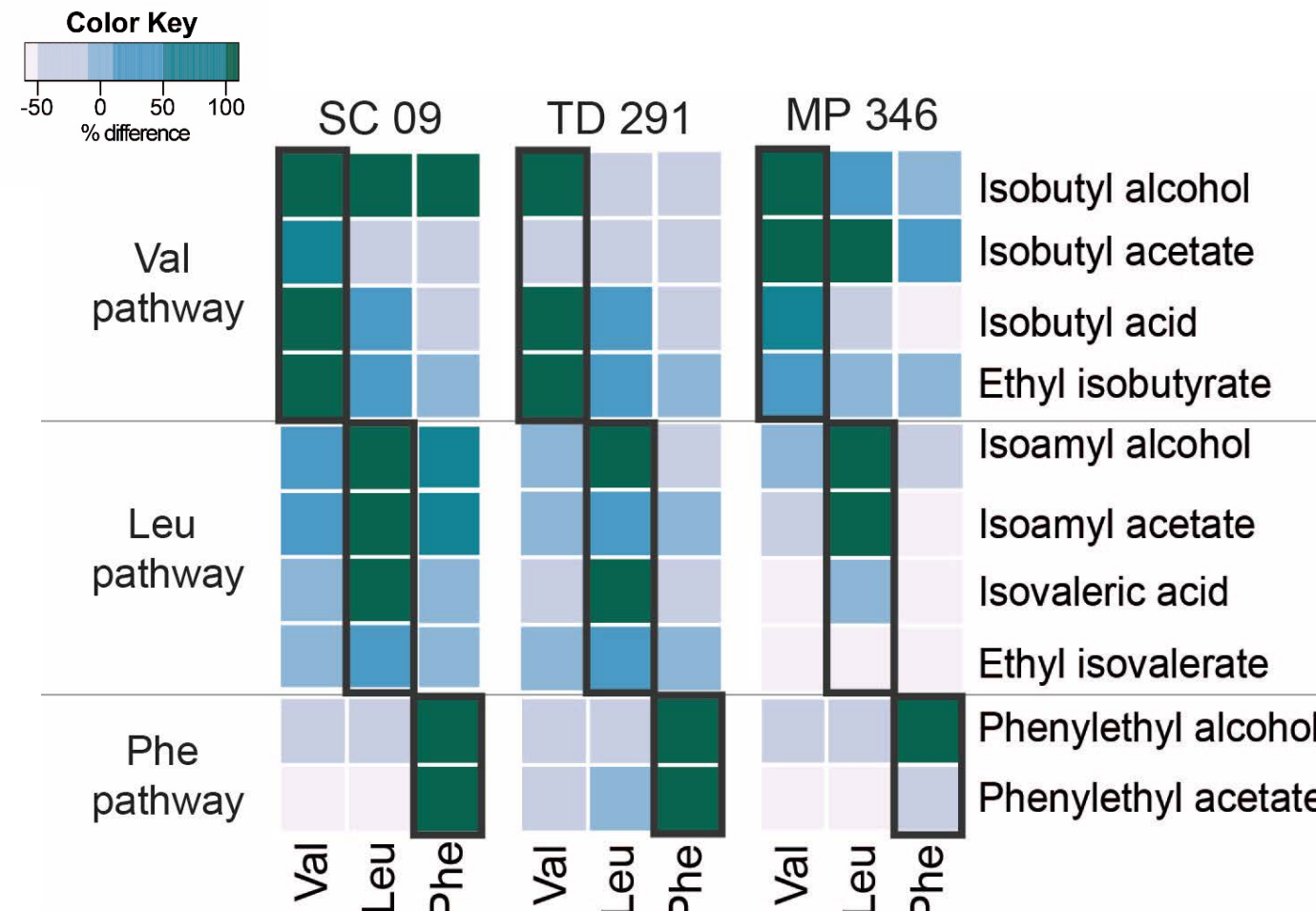
Percentage difference between the production of aroma compounds by TD 291 in complete medium vs on medium with only valine :



- Most of studied aroma compounds are produced irrespective of the nitrogen source
 - Compounds synthesized from MCC metabolites, regulated by total nitrogen availability
- Huge increase in volatiles molecules that directly derive from α-keto-isovalerate.
- Moderate increase of volatile compounds not derived from valine metabolism, as propanol
 - Impact of MCC

Impact of the nitrogen source on the production of some aroma through Ehrlich pathway

Percentage differences between the production on complete medium and on sole nitrogen source medium



- Overall, increase in the production of compounds derived from the nitrogen source
- Low ability of TD 291 to produce acetate esters except phenylethyl acetate
- In contrast, with MP 346 increase in the production of acetate esters except phenylethyl acetate. Low producer of acids and their ethyl esters derivatives

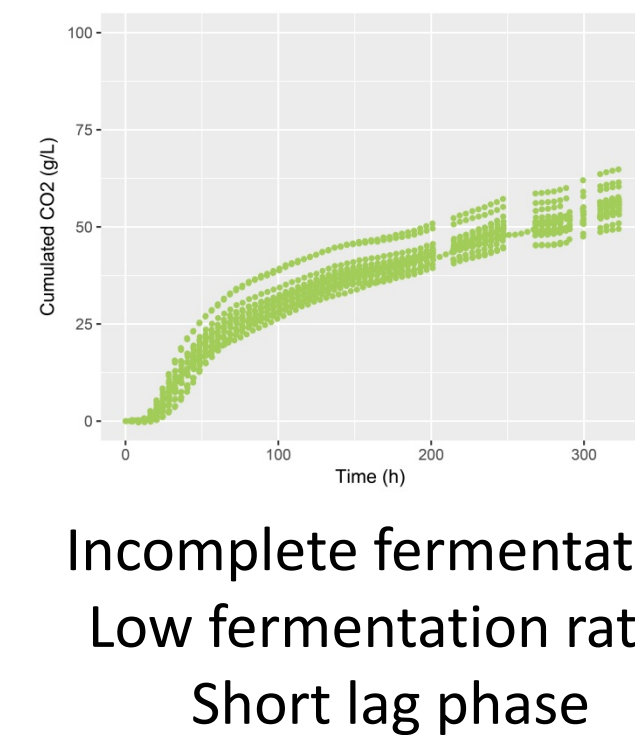
- Similar responses to the nature of the nitrogen sources for the 3 species
- However, some species specificities, likely related to their genetic background

Fermentation performances

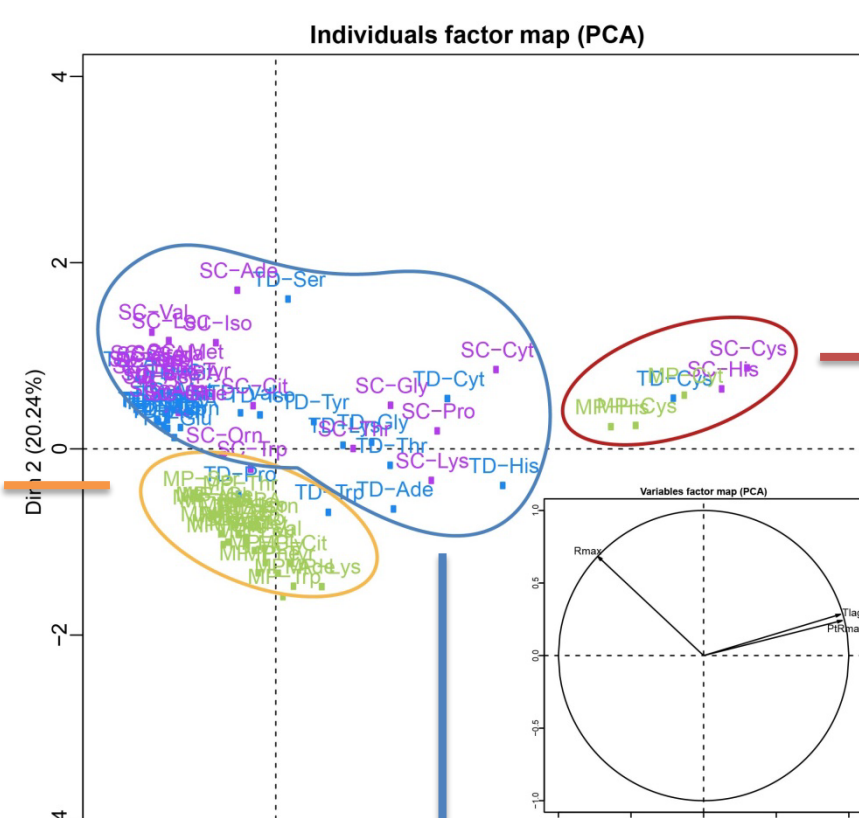
Fermentation profiles and kinetics parameters depend on both the strain and the nitrogen source

Principal component analysis of fermentation parameters : lag phase (Tlag), maximal fermentation rate (Rmax), and time to reach the Rmax (Trmax).

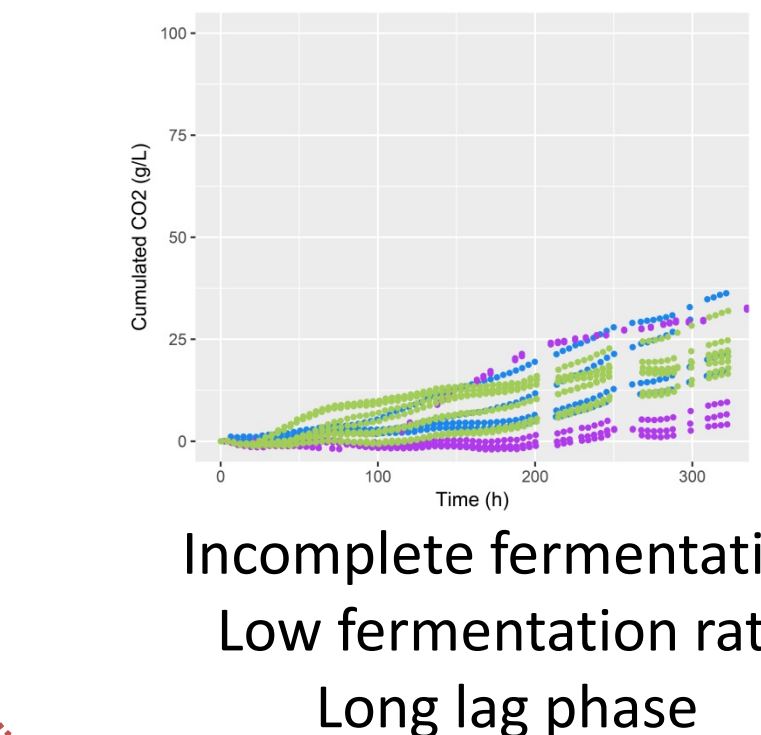
MP 346 : low performances regardless the nitrogen source



Incomplete fermentation
Low fermentation rates
Short lag phase

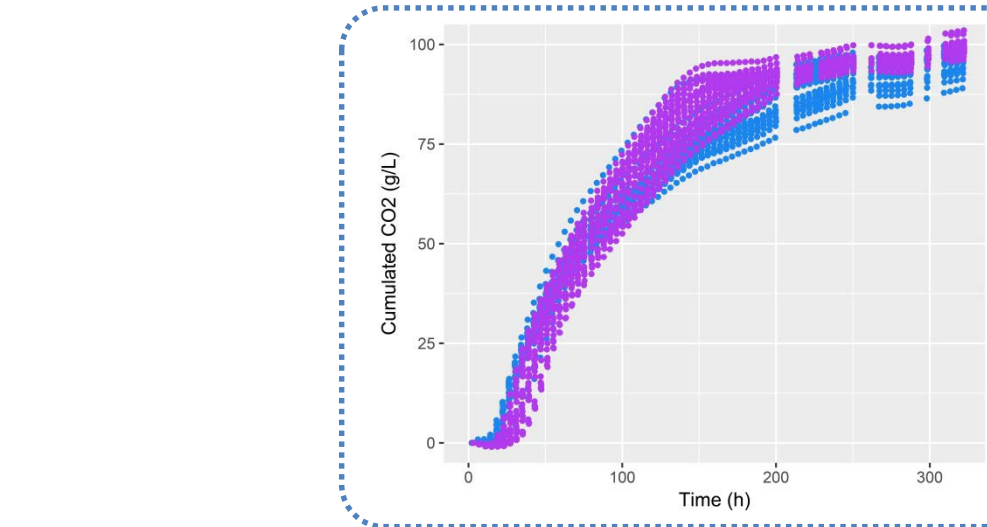


His, Lys, Cys : unable to support wine fermentation



Incomplete fermentation
Low fermentation rate
Long lag phase

SC 09 and TD 291 efficiently ferment sugars and most of the used nitrogen sources



Complete fermentation
High fermentation rates
Long lag phase

Efficiency of nitrogen sources to support wine fermentation

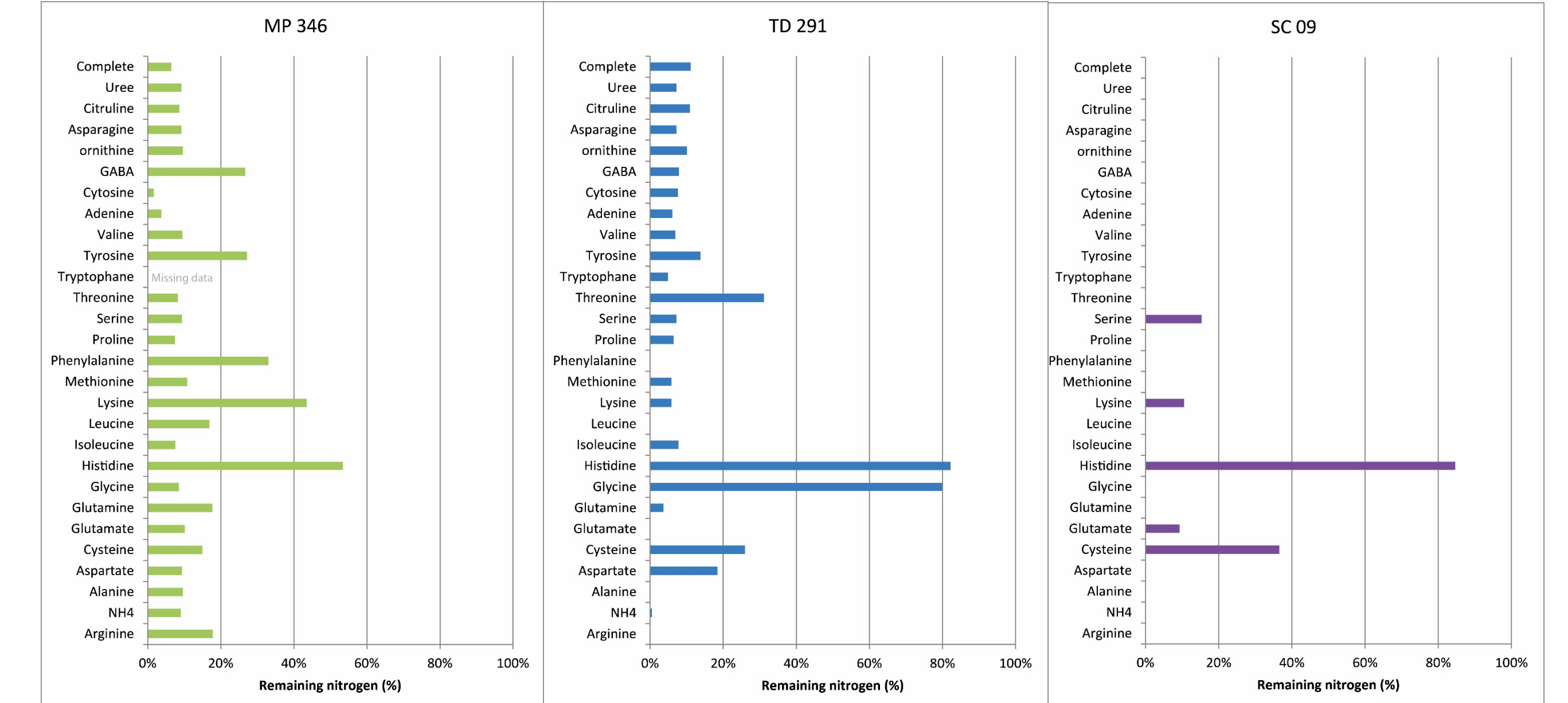
SC 09					TD 291					MP 346				
Ala	Met	Cyt	Trp	Cys	Ala	Leu	Cyt	Pro	Ade	Ala	NH4	Ade	Tyr	Cys
++	+	+	+	+	++	+	+	+	+	++	+	+	+	+
Arg	NH4	Gly	His		Arg	Met	Gly	Val		Arg	Orn	Cit		Cyt
Asn	Phe	Pro		Lys	Asn	NH4	Lys			Asn	Ser	Gly		His
Asp	Ser	Thr			Asp	Orn	Ser			Asp	Ure	Leu		Lys
GABA	Tyr	Ade			Cit	Phe	Thr			GABA	Thr	Phe		Trp
Gln	Ure	Cit			GABA	Ure	Trp			Gln		Pro		
Glu	Val	Iso			Glu	Ure	Tyr			Glu		Met		
Leu		Orn			Glu		Iso			Iso		Val		

++ : best nitrogen sources + : average sources + : poor sources. In bold, nitrogen sources in the same category for the 3 strains

- In contrast to MP 346, TD 291 and SC 09 are able to achieve fermentation using most of amino acids as sole nitrogen source
- Many similarities in nitrogen sources preferences between non-*Saccharomyces* strains and *S. cerevisiae*
- Some differences :
 - Phe supports more efficiently TD 291 and SC 09 fermentation than MP 346 fermentation
 - Trp can not be used by MP 346 to carry out fermentation unlike SC 09 and TD 291
 - Lys sustains a moderate fermentation by TD 291, but not for SC 09 and MP 346

Consumption of nitrogen sources

Percentage of residual nitrogen at the end of fermentation :



- SC 09 : exhaustion of most of the nitrogen sources except Ser (15%), Lys (11%), His (85%), Glu (9%) and Cys (39%).
- TD 291 : complete consumption of Phe, Glu, Arg and Ala. Limited ability (residual nitrogen > 80%) to assimilate His and Gly. Higher consumption of Cys, Glu and Ser than SC 09.
- MP 346 : consumption between 47 and 98% of initial nitrogen. Higher consumption of Cys, His and Ser than SC 09.
- Correlation between the amount of consumed nitrogen and fermentation performances. In particular, inability to sustain fermentation on poorly consumed nitrogen sources.

Distinct fermentative profiles were observed depending on the nitrogen source and the yeast strain. Yeasts had their own preferences in term of nitrogen sources that have shown diverse efficiency to sustain fermentative activity. Analysis of aroma compounds production according to the nitrogen source revealed a redistribution of carbon and nitrogen through the entire metabolism network, as the presence of a sole amino acid allow the production of all aroma compounds and not only those directly produced by the amino acid. Differences between the species have been highlighted, especially concerning the production of acetate esters. Nitrogen consumption is consistent with fermentation kinetics. These results provide new insights for the management of co-cultures of non-*Saccharomyces* and *S. cerevisiae*, emphasizing the importance of managing the nitrogen nutrition.

