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Impact of nitrogen sources on the fermentative kinetic of non -Saccharomyces yeasts

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Over the past 50 years, wine fermentation has been mainly controlled through the use of selected starter cultures of *S. cerevisiae*, to ensure the completion of fermentation and to avoid undesirable off-compounds. The non-*Saccharomyces* yeasts, predominant in grape juice, are rapidly outcompeted by *S. cerevisiae* during the process because of their poor adaptation to increasing concentrations of ethanol and the lack of oxygen. Even though these species were used to be considered as spoilage microorganisms for a long time, their potential to improve the sensory quality of wines is now recognized. However, the metabolic specificities of non-*Saccharomyces* yeasts remain ill-characterised, in particular their ability to catabolize the nitrogen compounds. It is thus critical to fill this shortcoming, nitrogen assimilation playing a key role in the fermentation process and the production of aroma compounds. Therefore, the aim of this study is to investigate the effect of various nitrogen sources on the fermentative kinetic of four non-*Saccharomyces* yeasts to determine their preferences in comparison with *S. cerevisiae*, in order to manage and optimize the implementation of these strains in co-culture with *S. cerevisiae*.

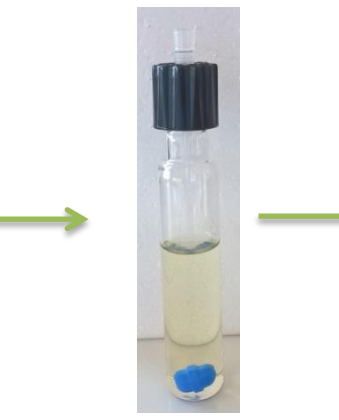
Material and methods

Fermentation in synthetic media with **only one nitrogen source** among 27 :

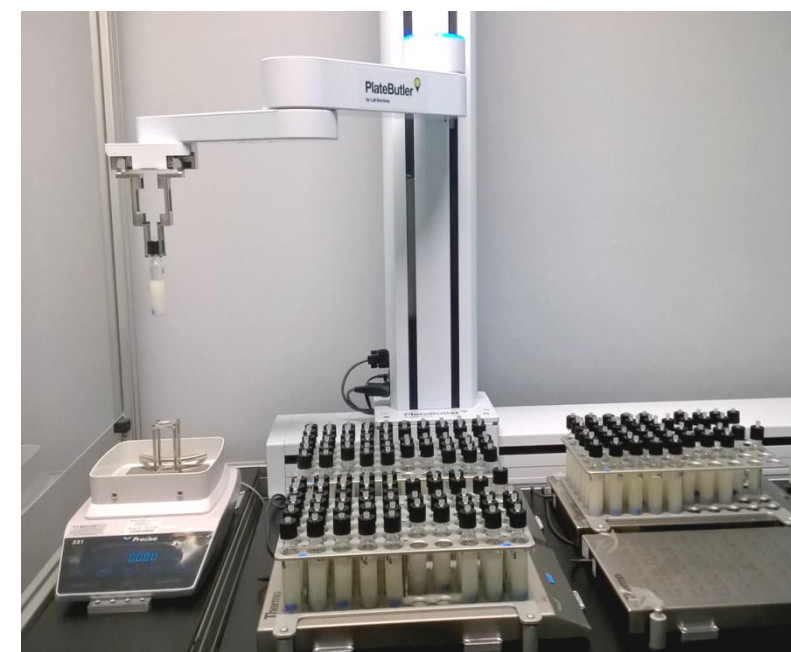
Adenine	Glutamine	Ornithine
Alanine	Glutamate	Phenylalanine
Arginine	Glycine	Proline
Aparagine	Histidine	Serine
Aspartate	Isoleucine	Threonine
Citruline	Leucine	Tryptophane
Cysteine	Lysine	Tyrosine
Cytosine	Methionine	Urea
GABA	NH ₄	Valine

Study of **5 yeasts** stains, naturally present on grapes :

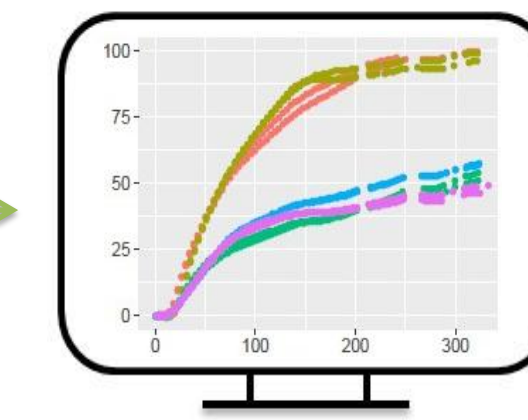
- *Torulospira delbrueckii* (TD 291)
- *Metschnikowia pulcherrima* (MP 346)
- *Metschnikowia fructicola* (strain A and B) (MFa and MFb)
- *Saccharomyces cerevisiae* (SC 09)



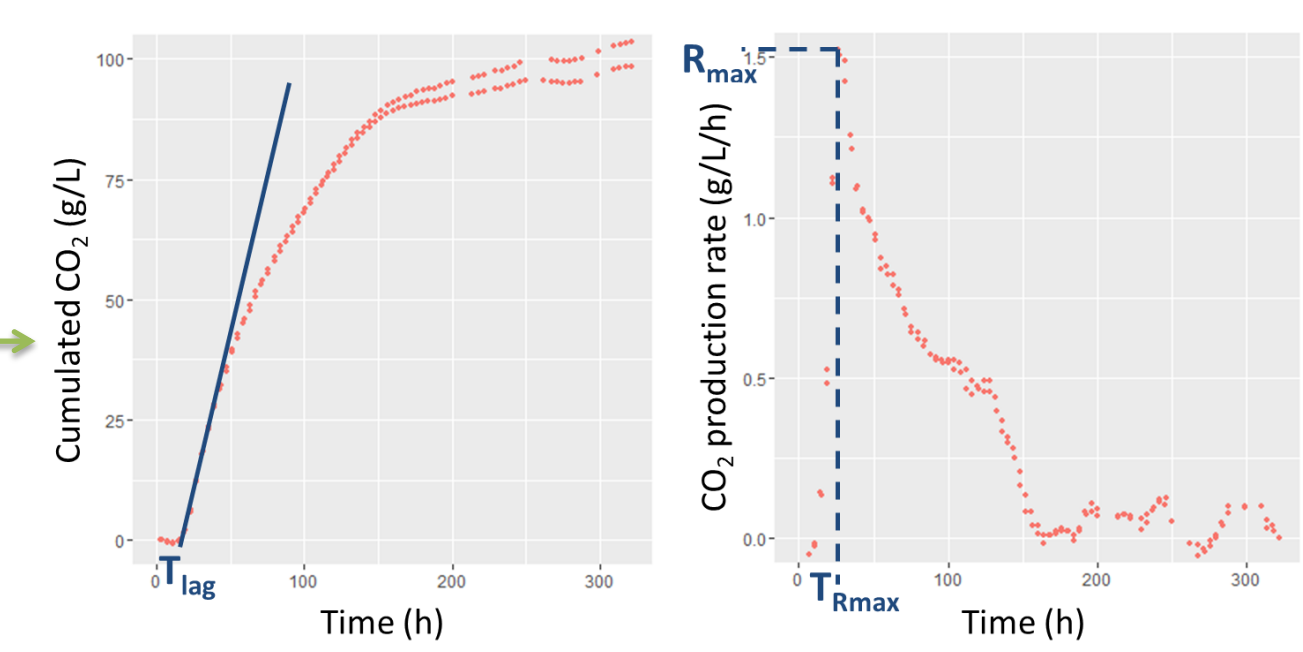
Tubes (15 mL)
1 AA per media



Phenoferm
Automatic weighing device



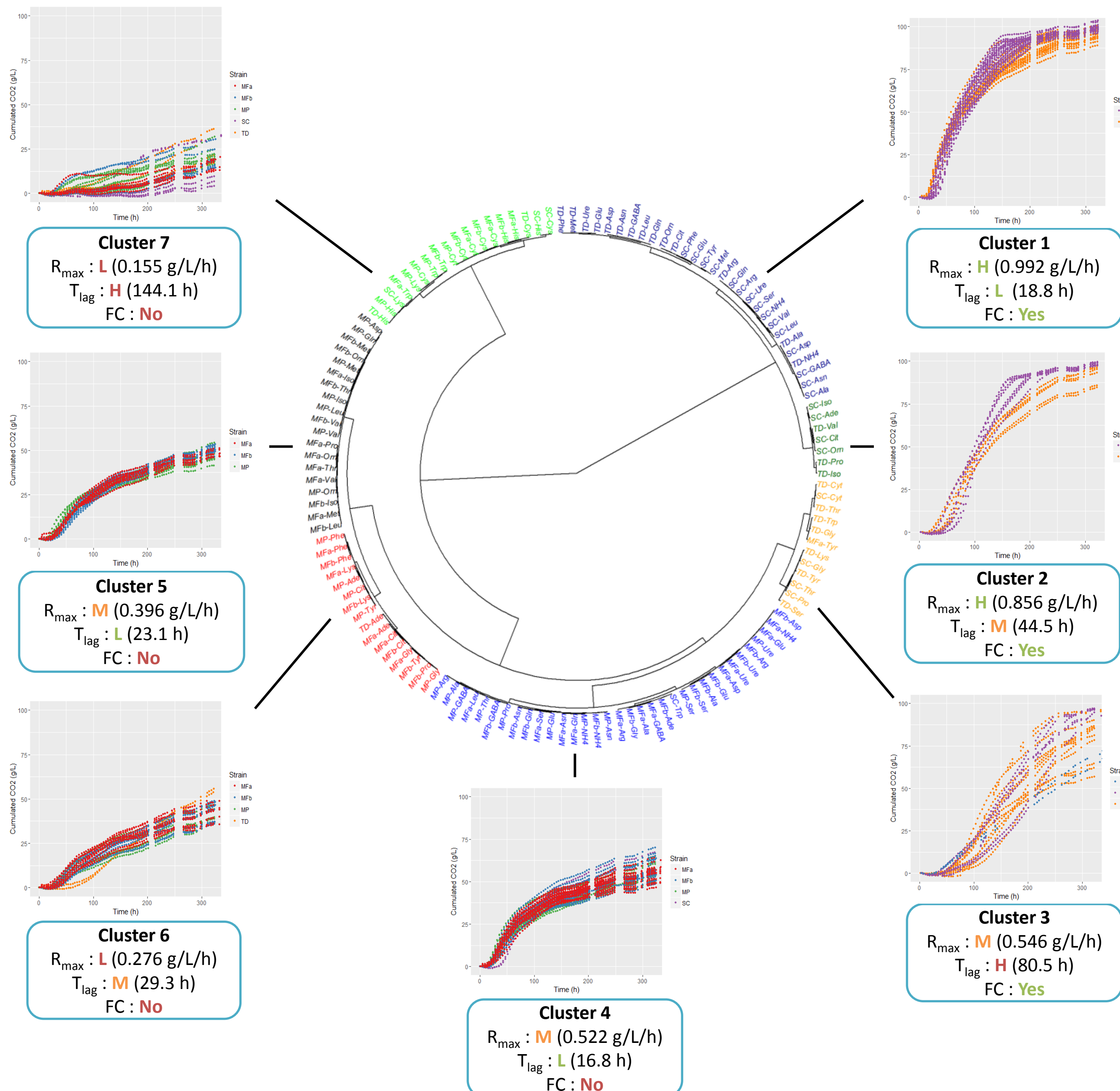
Data acquisition and treatment



Determination of characteristic parameters further used for statistical analysis

Nitrogen sources classification and efficiency

Clustering of fermentation profiles

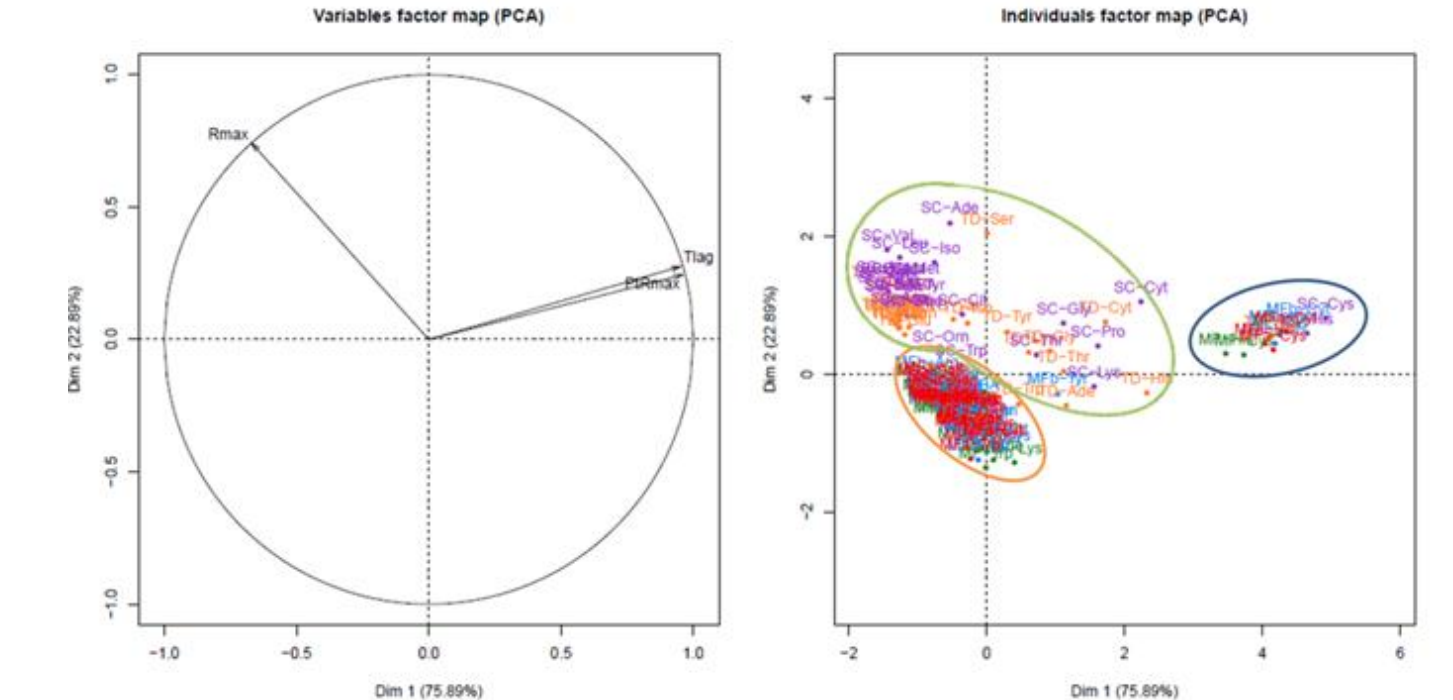


Individuals are grouped depending on their fermentation kinetic profiles in 7 clusters (fermentation with 200 mg/L of YAN, mean values of maximal fermentation rate (R_{max}) and lag phase (T_{lag}) are indicated in boxes, H: high, M: medium, L: low, FC: completion of fermentation, red: MFa, blue: MFb, green: MP, purple: SC, orange: TD)

PCA of characteristic parameters measured for 5 strains grown on sole nitrogen source

Important profile differences depending on the strain :

- Blue circle : no fermentation
- Orange circle : MP 346 and MF a et b, short lag phase, low R_{max}
- Green circle : TD 291 and SC 09, longer lag phase, higher R_{max}

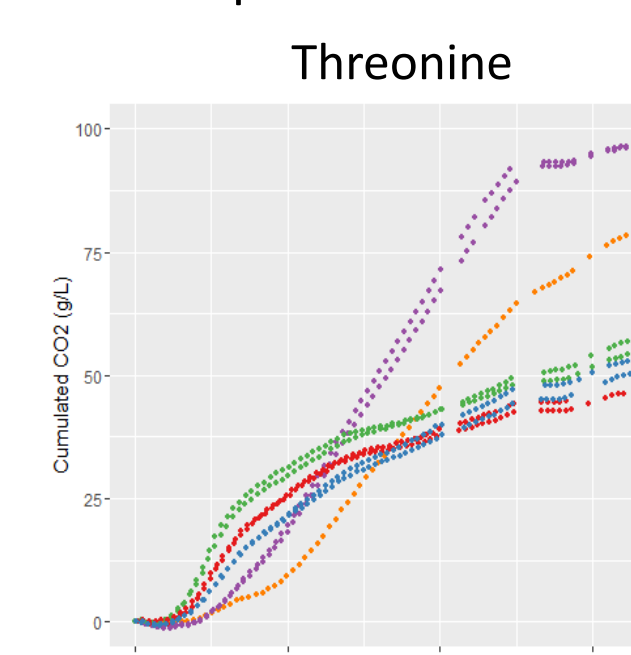


Efficiency of nitrogen sources depending on the strain

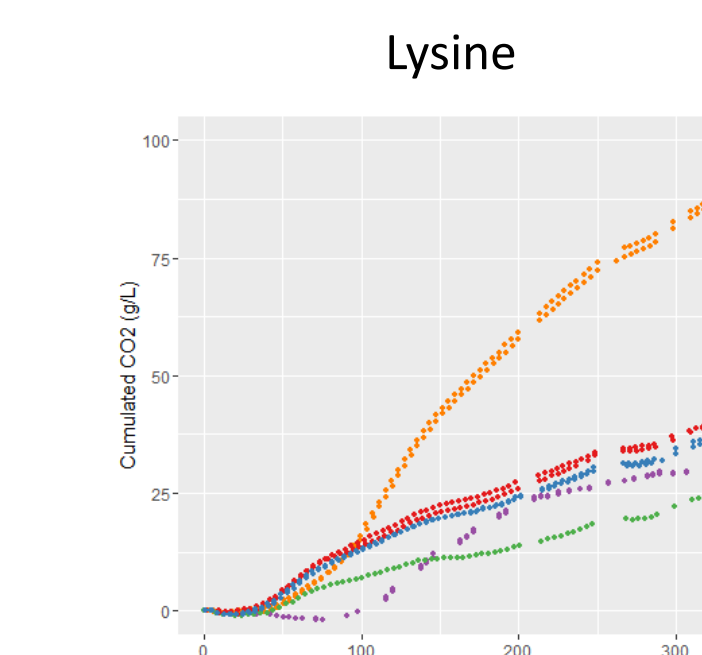
<i>T. delbrueckii</i>					<i>S. cerevisiae</i>					<i>M. fructicola</i>				
++	+	-	+	-	++	+	-	+	-	++	+	-	+	-
Ala	Ure	Cyt	Iso	Ade	Ala	Met	Cyt	Trp	Cys	Ala	NH ₄	Ade	Orn	Cys
Arg	Leu	Arg	Pro	Pro	Arg	NH ₄	Arg	His	His	Arg	Ser	Cit	Pro	Cyt
Asn	Met	Lys	Lys	His	Asn	Phe	Pro			Asn	Ure	Gly	Thr	His
Asp	NH ₄	Ser			Asp	Ser	Thr			Asp		Gly	Val	
Cit	Orn	Thr			GABA	Tyr	Ade			GABA		Phe	Trp	
GABA	Phe	Trp			Gln	Ure	Cit			Gln		Tyr		
Gln		Tyr			Glu	Val	Iso			Glu		Iso		
Leu		Val			Leu		Orn			Leu		Met		

++ : best nitrogen sources + : average sources - : poor sources. In bold, nitrogen sources in the same category for the 5 strains

- Same classification for the three *Metschnikowia*
- SC 09 and TD 291 have mostly the same preferences
- Exceptions : TD



- Shorter lag phase for *Metschnikowia*
- Threonine more favourable for SC than TD



- Almost no fermentation for SC
- Different pattern between MP and MF
- TD is able to ferment on lysine

Different fermentation profiles depending on the strain and the nitrogen source

Incidence of the nitrogen source :

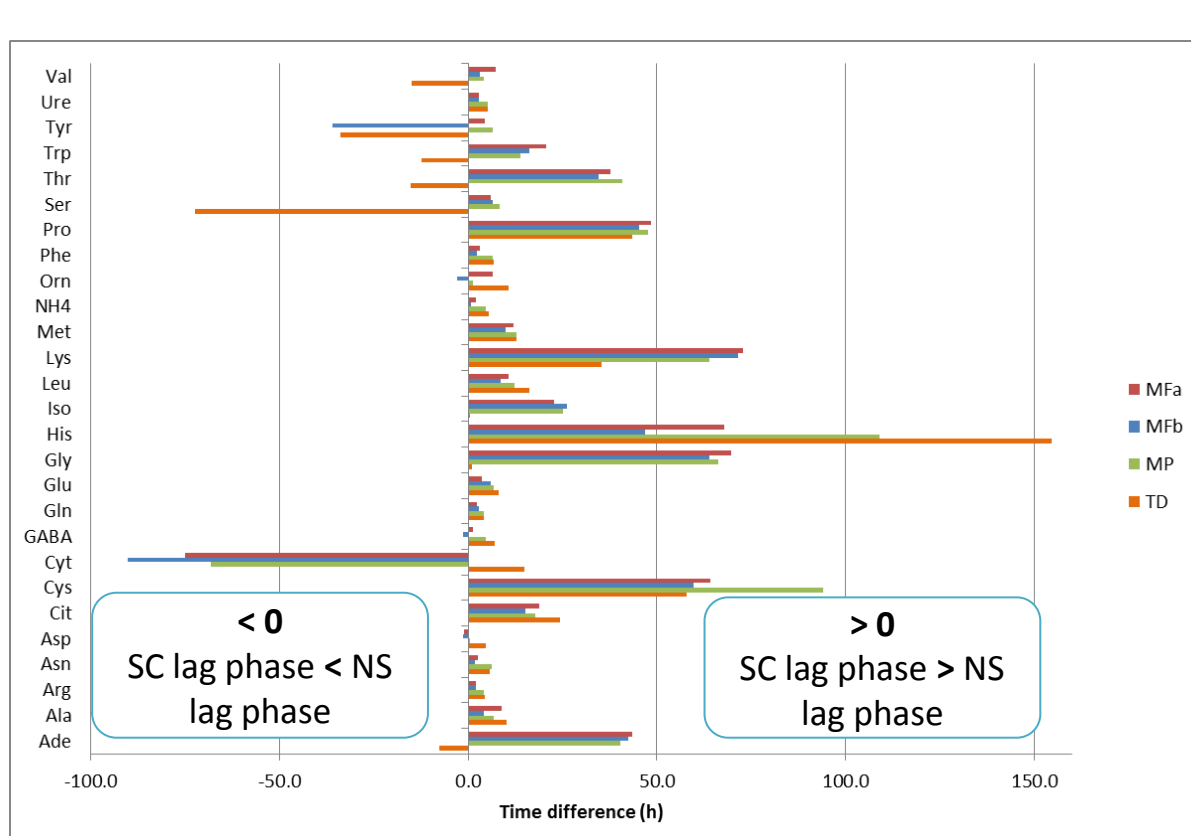
- histidine and cysteine = no fermentation
- arginine, glutamine, glutamate, ammonium = efficiently used regardless of the strain

Incidence of the strain = two groups of strains having the same profile :

- SC and TD : best adapted for alcoholic fermentation, high R_{max}, complete the fermentation most of the time
- MF and MP : low R_{max}, do not complete the fermentation but short lag phase

Application for co-culture management

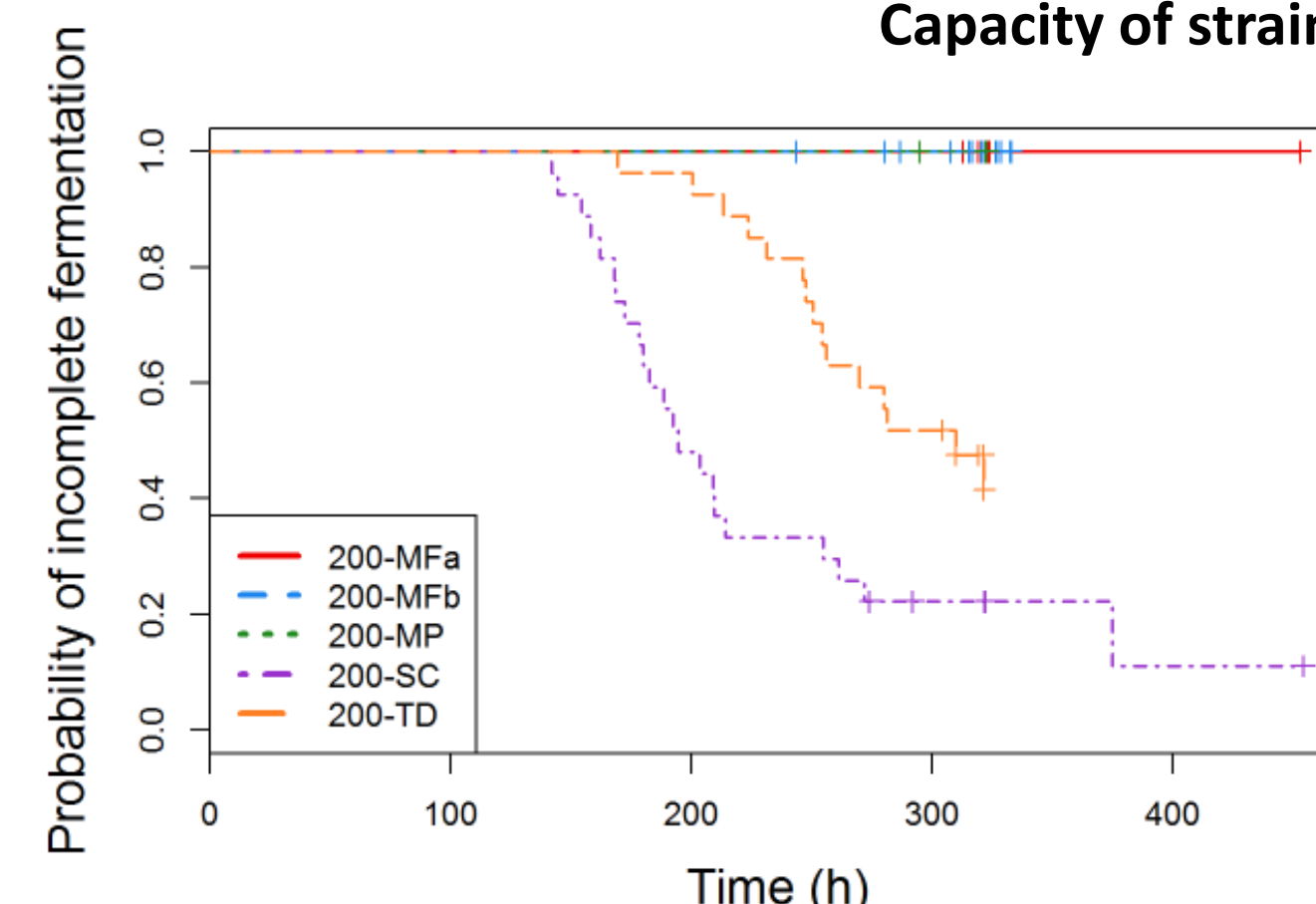
Impact of nitrogen nature on the lag phase



- Representation of *S. cerevisiae* lag phase minus non-*Saccharomyces* strains lag phase = when the difference is > 0, SC lag phase is longer than the lag phase of the other strain

- Under most conditions, shorter lag phase for non-*Saccharomyces*
- Non-*Saccharomyces* start to consume nitrogen before *S. cerevisiae* = competition for nitrogen in sequential or co-inoculation ?

Capacity of strains to complete the fermentation



- Representation of the probability of incomplete fermentation (final CO₂ production < 90 g/L)

- MP and MF are not able to complete the fermentation regardless of the nitrogen source
- TD is less effective than SC, but is able to complete the fermentation in many conditions = consumption of nutrients in the medium (sugar, nitrogen, ...)
- Competition between TD and SC in co-culture ?

Distinct fermentative profiles were observed depending on the nitrogen source and the yeast strain. Yeasts had their own preferences in term of nitrogen sources, which have shown diverse efficiency to sustain fermentative activity. It would be interesting to complete these results with the analysis of nitrogen impact on yeast growth, to provide a comprehensive overview of the capabilities of non-*Saccharomyces* yeasts to efficiently metabolize nitrogen compounds. Overall, for most nitrogen sources, non-*Saccharomyces* yeasts exhibit a shorter lag phase than *S. cerevisiae* and a lower fermentation rate. These results provide new insight for the management of sequential or co-inoculation of non-*Saccharomyces* and *S. cerevisiae*, emphasizing the importance of managing the nitrogen nutrition throughout the process.

