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## DECIPHERING YEAST-YEAST INTERACTIONS IN OENOLOGICAL FERMENTATION

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### Text of the abstract:

In natural or anthropized environments, microbial species are part of an ecosystem and interact in a complex network in advantageous or disadvantageous way. Until recently, process optimization in agriculture or food processing was mostly based on the selection of single strains. However, this paradigm is now being challenged and the scientific community is increasingly seeking to exploit and optimize consortia consisting of several strains. Indeed, many studies have shown that more diverse anthropized environments have many advantages in terms of resilience, disease resistance or yield of production (Barot et al. 2017). Efforts are now being made to design optimal consortia of various species and strains whose interactions will be exploited to maximize a given criterion. In this context, the so-called ‘transgressive’ interactions, are the most interesting to exploit. Transgressive interactions are observed when a mixture of entities has a better (or lower) performance than the best (or worst) of the entities cultivated separately. These transgressive interactions, which are the cause of ‘over yielding’, are already exploited and studied in agriculture (Barot et al. 2017), but are still very little exploited in microbial ecosystems.

Microbial ecosystems are widely exploited in anthropogenic environments. They are used in pollution control, environmental protection, agriculture, pharmacology and food production (Ciani et al. 2015). Among the food ecosystems, oenology that has great importance, both economic and societal use regularly the addition at the beginning of fermentation ‘starters’ composed of selected yeasts. It is estimated that 80% of the oenological fermentations in the world are conducted with their use (Marsit et al. 2015). Most often these fermentation starters are composed of a single yeast strain of the species *Saccharomyces cerevisiae* selected for its ability to complete the fermentation. However, in recent years and under the pressure of consumers for more aromatic wines, multi-species starters have emerged which most often combine, a strain of *S. cerevisiae* allowing to complete the fermentation and a strain of a different species, often from a different genus than *Saccharomyces*, bringing a greater variety of flavours (Ivey et al. 2013). Nevertheless, the composition and the protocol of inoculation of these multi-strains starters are still very empirical and only based on input/output balance without taking into account the dynamics of the microbial populations and their interactions. This lack of knowledge about the yeast-yeast interactions prevents improving a reasoned design of multi-strain starters (Song et al. 2014).

To resolve this problem, we decide to precisely follow population dynamics and metabolite productions during oenological fermentations realized by unique or mixed populations of yeasts. This detailed analysis of the fermentation dynamics will help to better understand the mechanisms of interactions and the influence of yeast-yeast interactions on the metabolic composition of the final product.

### Material and Methods

We performed fermentations in oenological conditions (200 g/L of sugars, 200 mg/L of assimilable nitrogen) mediated by one or two species of yeasts. *S. cerevisiae* was tested against 5 different species of yeasts: *Metschnikowia pulcherrima*, *Metschnikowia fructicola*, *Hanseniaspora opuntiae*, *Hanseniaspora uvarum* and *Torulaspora delbrueckii*. We inoculate  $9 \cdot 10^5$  cells/mL of the non-saccharomyces species and  $1 \cdot 10^5$  cells/mL of *S. cerevisiae*. We also performed isolated cultures of each specie inoculated at  $10^6$  cells/mL. During the fermentation process, CO<sub>2</sub> production was online monitored (every 20 minutes). Population density, viability and proportion of *S. cerevisiae* in mixed cultures were offline measured every 12 hours during the first week and every 24h during the second week with a flow cytometer. At the same time scale, consumption of glucose and fructose, production of ethanol, glycerol, succinate, acetate and alpha keto-glutarate were measured by HPLC. Finally, the residual concentrations of nitrogen compounds (ammonium and amino-acids) were evaluated at the end of the fermentation. All these data were analysed to identify the intensity of interactions and the mechanisms underlying them.

## Results

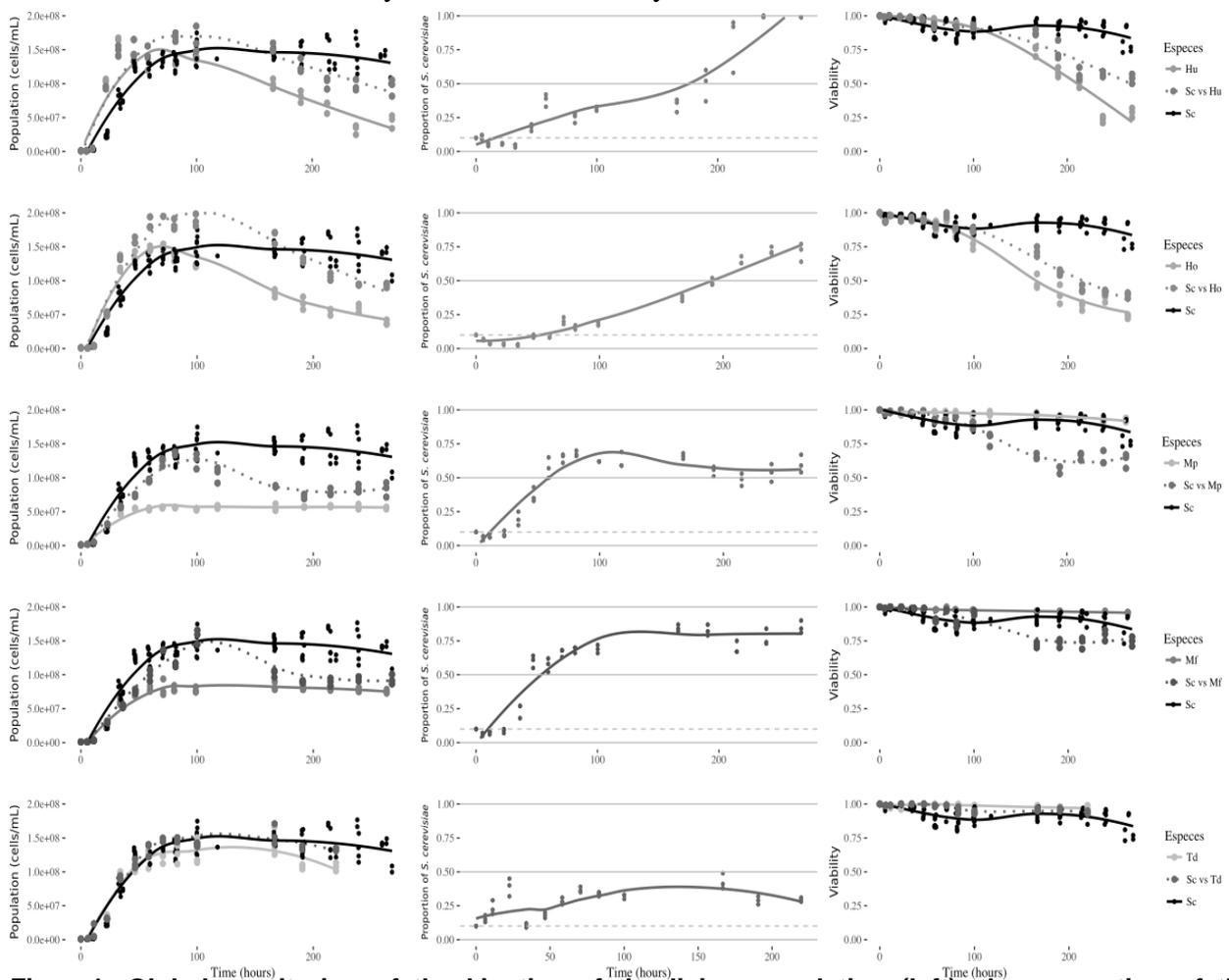
In this short paper, we analysed competitions separately in function of the non-saccharomyces species that is involved and only compare them to the *S. cerevisiae* species that is our central reference we will also leave aside the detail of the consumption of resources. In the case of *Torulaspora delbrueckii* (figure 1 bottom line), the dynamic in both isolated and mixed cultures are a very similar. We have almost the same growth rate, carrying capacity and viability. Furthermore, the viability is almost equal to 100% throughout the fermentation process indicating that there is no mortality in our culture conditions. Yet the proportion of *S. cerevisiae* increase during the fermentation passing from 10% to around 25% of the culture, indication a slightly better growth rate of *S. cerevisiae*. These measures indicate a simple case of resource competition of two species with very similar behaviours.

The competitions with the two *Hanseniaspora* species have a similar dynamic (figure 1 first and second line). The two *Hanseniaspora* isolated cultures have an exponential growth phase followed by a long phase of mortality where the viability decreases up to 25% of living cells. The mixed cultures between *Hanseniaspora* species and *S. cerevisiae* have an intermediate dynamic between both isolated culture with a similar mortality that explains the increase in frequency until an almost fixation of *S. cerevisiae* after 200h of fermentation. These dynamics indicate a simple competition for resources as with *T. delbrueckii* but this time with differential mortalities between the *Hanseniaspora* species and *S. cerevisiae*.

The competition with *Metschnikowia* species is different (figure 1, lines 3 and 4). In the isolated cultures, there is a phase of exponential growth followed by a stationary phase where the viability remains constant during the entire fermentation. On the opposite, in the mixed cultures, we observed a decrease in viability during the stationary phase. It is not clear if this decrease in viability is linked with the death of both species or only one. The fact that during the stationary phase the frequency of *S. cerevisiae* remains constant seems to indicate that both species are equally affected. Further investigation will be necessary to clarify the mechanism undergoing the mortality that only appears in the mixed fermentations.

## Conclusion

This work makes it possible a better understanding of yeast-yeast interactions in oenological conditions. It seems that most interactions are indirect and mediated by the competition for resources. This observation leads to the possibility to predict with mathematical models and with data from isolated cultures only, the fermentation dynamics of mixed cultures. In the future, using



**Figure 1:** Global monitoring of the kinetics of the living population (left), the proportion of the *S. cerevisiae* in mixed cultures (center) and the viability (right) across the fermentation. All points represent a sample and the curve lines a smoothing of the data. The full lines are for isolated cultures and the dashed lines for mixed cultures. The light gray colours represent pure culture of 'non-*Saccharomyces*' strains and the darker, to the corresponding culture in competition with *S. cerevisiae*. The isolated cultures of *S. cerevisiae* are represented in black. *Sc*, *Mp*, *Mf*, *Ho*, *Hu*, *Td*, *Sc vs Mp*, *Sc vs Mf*, *Sc vs Ho*, *Sc vs Hu*, *Sc vs Td* correspond respectively to cultures with: isolated *S. cerevisiae*, isolated *M. pulcherrima*, isolated *M. fructicola*, isolated *H. opuntiae*, isolated *H. uvarum*, isolated *T. delbrueckii*, *M. pulcherrima* mixed with *S. cerevisiae*, *M. fructicola* mixed with *S. cerevisiae*, *H. opuntiae* mixed with *S. cerevisiae*, *H. uvarum* mixed with *S. cerevisiae* and *T. delbrueckii* mixed with *S. cerevisiae*.

such models will allow optimization of yeast consortia: frequencies of inoculation, possibilities to perform simultaneous or sequential inoculations.

## Keywords

Oenology, Fermentation, yeast-yeast interactions, population dynamics, non-saccharomyces species

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