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Non-Saccharomyces yeast nitrogen consumption and metabolite production during wine fermentation

P. Seguinot, V. Englezos, G. Bergler, P. Brial, A. Ortiz-Julien, M. Brulfert, C. Camarasa, A. Bloem
Context: toward the diversification of enological yeasts

- Wide diversity of yeast species at the beginning of the fermentation → But domination of *S. cerevisiae* at the end of FA

- Use of active dry yeasts to control the process

- Some of the non-*Saccharomyces* yeasts have interesting properties

- Association of a non-*Saccharomyces* strain with *S. cerevisiae*:
  - Non-*Saccharomyces*
    - Aroma production
    - Do not complete the fermentation
  - *S. cerevisiae*
    - Fermentation completion

Interactions: nutrients competition

- Contribution of *M. pulcherrima* Flavia® for the production of aromas ?
- Assessment of nutrient requirements to ensure alcoholic fermentation ?
**M. pulcherrima Flavia® in sequential inoculation with S. cerevisiae Lalvin QA23®**

- **Experimental conditions:**
  - In SM with thiol precursors
  - **Fermentation progress**
    - T=0h: inoculation of *M. pulcherrima Flavia®*
    - T=48h: inoculation of *S. cerevisiae Lalvin QA23®*
  - Aroma analysis by GC/MS

- **Results: fermentative aroma compounds**

- **Characteristic profile of fermentative aromas production, with increased production of acetate esters derived from branched higher alcohols**
  - diversification of aroma profile of wines
**M. pulcherrima Flavia®** in sequential inoculation with *S. cerevisiae* Lalvin QA23®

- Results: thiols

- Strong increase in the production of thiols in seq. inoculation with *M. pulcherrima* Flavia®

![Graph showing increase in thiols](image)
- Phenotypic diversity of non-\textit{Saccharomyces} yeasts for the production of aromas to be exploited

- Management of nitrogen important in sequential inoculation for the alcoholic fermentation process. Co-cultures result in interactions between the yeasts with effect on the quality of the final product, on the production of positive aromas.

\begin{itemize}
  \item Nitrogen sources prematurely consumed
  \item \textit{M. pulcherrima} Flavia® nitrogen consumption characteristics
\end{itemize}

\begin{itemize}
  \item 28\% of yeast assimilable nitrogen consumed by \textit{M. pulcherrima} in 48h: mainly \textit{Glu, Gln and} amino acids branched
  \item Lower implantation of S.c in sequential inoculation
\end{itemize}
Thank you for your attention

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Vasilis,
Guillaume

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Anne Ortiz-Julien

Michel Brulfert
**M. pulcherrima Flavia®**: A high ability to secrete hydrolytic enzymes

- Release of hydrolytic enzymes into the must, such as β-lyase which involved in the cleavage of thiol precursors → diversification of aroma profile

**Chart**

- **β-lyase U/ml**
  - S. cerevisiae (control)
  - Sequential inoculation (M.p et S.c)

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>S. cerevisiae (control)</th>
<th>Sequential inoculation (M.p et S.c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>0.02</td>
<td>S. cerevisiae</td>
</tr>
<tr>
<td>12</td>
<td>0.14</td>
<td>S. cerevisiae</td>
</tr>
<tr>
<td>24</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>0.10</td>
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</tr>
<tr>
<td>72</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>96</td>
<td>0.02</td>
<td></td>
</tr>
</tbody>
</table>

**Thiol precursors**
- NH₄, amino acids or peptides
Impact of must characteristics on *M. pulcherrima* Flavia® performances

**Thiols**

- 3MH and 4MMP : same shape = same liberation pathway
- 3MHA : different shape, close to acetate esters = similar production pathway

- Quadratic effect of nitrogen on the release of 3MH and 4MMP
- Positive effect of nitrogen on the production of 3MHA

*3MHA production using similar mechanisms than the other acetate esters*