Supplementary Material

**Analysis of the NCR mechanisms in *Hanseniaspora vineae* and *Saccharomyces cerevisiae* during winemaking**

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**Supplementary Table 1.** Synthetic must composition used in the present work.

**Synthetic must composition (140 mg N/L)**

 1 L

Glucose 100 g

Fructose 100 g

Citric acid 5 g

Malic acid 0.500 g

Tartaric acid 3 g

KH2PO4  0.750 g

K2SO4 0.500 g

MgSO2 7 H2O 0.250 g

CaCl2 2 H2O 0.155 g

NaCl 0.200 g

Nitrogen (140 mg N/L)

 NH4Cl (56 mg N/L) 0.214 g

 Amino acid solution (84 mg N/L) 4.78 mL

Oligo-elements solution 1 mL

Vitamins solution 10 mL

Anaerobic factors 1 mL

**Vitamins stock solution**

 1 L

Myo-inositol 2 g

Pantothenate calcium 0.150 g

Thiamine hydrochloride 0.025 g

Nicotinic acid 0.200 g

Pyridoxine 0.025 g

Biotine  3 mL (from a stock of 100 mg/L)

**Oligo-elements stock solution**

 1 L

MnSO4 H2O 4 g

ZnSO4 7 H2O 4 g

CuSO4. 5 H2O 1 g

KI 1 g

CoCl2 6 H2O 0.4 g

H3BO3  1 g

(NH4)6Mo7O24  1 g

**Anaerobic factors stock solution**

 0.100 L

Ergosterol 1.5 g

Oleic acid 0.5 mL

Tween 80 50 mL

Ethanol (absolute) until 100 mL

**Supplementary Table 2.** Ammonium content and amino acid stock solution content expressed as g L-1 and the corresponding nitrogen and YAN concentration in synthetic must in mg N L-1.

|  |  |  |  |
| --- | --- | --- | --- |
| **Amino acid** | **g L-1\*** | **mg N L-1** | **mg YAN/L**  |
| Asp | 4.42 | 2.22 | 2.22 |
| Glu | 11.96 | 5.44 | 5.44 |
| Ser | 7.80 | 4.97 | 4.97 |
| Gln | 49.92 | 45.76 | 22.88 |
| His | 3.38 | 1.46 | 1.46 |
| Gly | 1.82 | 1.62 | 1.62 |
| Thr | 7.54 | 4.24 | 4.24 |
| Arg | 36.79 | 42.45 | 14.15 |
| Ala | 14.56 | 10.95 | 10.95 |
| Tyr | 1.95 | 0.72 | 0.72 |
| Cis | 2.08 | 1.15 | 1.15 |
| Val | 4.42 | 2.53 | 2.53 |
| Met | 3.12 | 1.40 | 1.40 |
| Trp  | 17.42 | 5.71 | 5.71 |
| Phe | 3.77 | 1.53 | 1.53 |
| Ile | 3.25 | 1.66 | 1.66 |
| Leu | 4.81 | 2.46 | 2.46 |
| Lys | 1.69 | 1.55 | 0.77 |
| Pro | 59.93 | 0,00 | 0.00 |
| **Total aas** |  | 137.83 | 85.88 |
| **Ammonia (NH4Cl)** | 0.214 | 56.00 | 56.00 |
| **Total YAN** |  |  | 141.88 |
| **Total N** |  | 193.83 |  |

\* To achieve a final concentration of 140 mg YAN/L (190 mg N/L), 4.78 mL of amino acid stock solution is added to 1 L of synthetic must.

**Supplementary Table 3.** Homology ofNCR related proteins between *H. vineae* and *S. cerevisiae* EC1118. Genes highlighted in bold are the ones which expression has been analyzed in this study.

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| --- | --- | --- | --- | --- | --- |
| Gene name | Systematic name | Pfam domain | Putative orthologousin *H. vineae* | Aminoacidic similarity  | Single copy gene in *H. vineae* |
| ***AGP1***  | YCL025C | AA\_permease | g1661.t1;g1666.t1 | 50.43 %; 50.77 % | no |
| ***GAP1*** | YKR039W | AA\_permease | g4653.t1 | 67.06 % | no |
| ***MEP2***  | YNL142W | Ammonium\_transp;Ammonium transporter AmtB-like domain | g3765.t1 | 60.82 % | yes |
| ***PUT2***  | YHR037W | Aldehyde dehydrogenase domain | g905.t1 | 67.50 % | yes |
| *GAT1* | YFL021W | GATA zinc finger; Fungal protein of unknown function | g3143.t1 | 51.38% | yes |
| *GLN3* | YER040W | GATA zinc finger | g1456.t1 | 36.64% | yes |
| *GZF3* | YJL110C | GATA zinc finger | g1991.t1 | 45.23% | yes |
| *DAL80\** | YKR034W | - | - | - | - |

\**DAL80* is not present in *H. vineae*

**Supplementary Table 4.** Gene expression of *AGP1*, *GAP1*, *MEP2* and *PUT2* during the first 48 h of fermentation. Gene expression (2^-ΔΔCt) of each gene at different time points was determined during the first 48 h, considering 4 h after inoculation as the reference time. The values are expressed as the mean Log10 relative gene expression. The resulting Log10 2^-ΔΔCt values were statistically analyzed using ANOVA and Tukey’s post-test. Different letters indicate significant differences in gene expression within each strain, p < 0.05.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Time (hours) | *AGP1* | *GAP1* | *MEP2* | *PUT2* |
| *H. vineae* T02/5AF | 8 | 0,037a±0,023 | -0,543a±0,146 | 0,080a±0,332 | -0,133a±0,152 |
| 12 | -0,557a,b±0,206 | -0,734a±0,016 | 0,080a±0,200 | -0,134a±0,016 |
| 16 | -0,456a,b±0,185 | -0,318a±0,197 | 0,039a±0,251 | 0,533a,b±0,403 |
| 20 | -0,506a,b±0,358 | -0,437a±0,308 | 0,235a±0,294 | 0,703a,b±0,338 |
| 24 | -0,782a,b±0,085 | 0,434a,b±0,076 | 0,573a,b±0,109 | 1,236b,c±0,018 |
| 30 | -1,191b±0,621 | 1,447b,c±0,661 | 0,914a,b±0,604 | 1,061b,c±0,126 |
| 36 | -1,019a,b±0,621 | 1,722c±0,371 | 1,183a,b±0,159 | 1,699c±0,116 |
| 48 | -0,297a,b±0,180 | 2,014c±0,106 | 1,474b±0,266 | 1,744c±0,177 |
| *H. vineae* T02/19AF | 8 | -0,452a±0,346 | -0,669a±0,033 | -0,535a±0,220 | -0,243a±0,130 |
| 12 | -0,515a±0,117 | -0,666a±0,063 | -0,629a±0,118 | -0,103a±0,019 |
| 16 | -0,521a±0,422 | -0,766a±0,012 | -0,526a±0,220 | 0,038a,b±0,436 |
| 20 | -0,602a±0,210 | -0,573a±0,245 | -0,477a±0,351 | 0,243a,b±0,036 |
| 24 | -0,746a,b±0,255 | 0,236b±0,022 | 0,271a,b±0,053 | 0,471a,b,c±0,051 |
| 30 | -1,437a,b ±0,238 | 0,560a,b±0,173 | 0,870b±0,022 | 0,716b,c±0,019 |
| 36 | -1,762b±0,131 | 0,642a,b±0,102 | 0,524b±0,431 | 1,105c±0,308 |
| 48 | -1,269a,b±0,201 | 0,738a±0,011 | 0,484b±0,066 | 1,111c±0,099 |
| *S. cerevisiae* QA23 | 8 |

|  |
| --- |
| 0,106a±0,040 |

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|  |
| --- |
| -0,887a±0,224 |

 | -0,330a±0,177 | -0,227a±0,097 |
| 12 | -0,018a,b±0,003 | -0,737a±0,437 | -0,442a±0,645 | -0,014a±0,321 |
| 16 | -0,541a,b,c±0,116 | 0,086a,b±0,099 | -0,149a±0,522 | 0,108a±0,519 |
| 20 | -0,593a,b,c±0,010 | 0,210b,c±0,077 | 0,146a,b±0,183 | 0,031a±0,044 |
| 24 | -0,061a,b±0,223 | 0,590b,c±0,188 | 0,513a,b±0,241 | 0,797a,b±0,329 |
| 30 | -1,022c±0,089 | 0,962c,d±0,100 | 0,565a,b ±0,022 | 1,603b,c±0,036 |
| 36 | -0,705b,c±0,309 | 1,098c,d±0,162 | 0,809a,b ±0,252 | 1,846c±0,171 |
| 48 | -0,370a,b,c±0,293 | 1,567d±0,282 | 1,385b±0,204 | 1,922c±0,150 |

