1 **Bioprotection by non-*Saccharomyces* yeasts in oenology: evaluation of O_2 consumption and impact on**
2 **acetic acid bacteria**

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ABSTRACT

Bioprotection by yeast addition is increasingly used in oenology as an alternative to sulfur dioxide (SO_2). Recent studies have also shown that it is likely to consume dissolved O_2. This ability could limit O_2 for other microorganisms and the early oxidation of the grape must. However, the ability of yeasts to consume O_2 in a context of bioprotection was poorly studied so far considering the high genetic diversity of non-*Saccharomyces*. The first aim of the present study was to perform an O_2 consumption rate (OCR) screening of strains from a large multi species collection found in oenology. The results demonstrate significant inter and intra species diversity with regard to O_2 consumption. In the must *M. pulcherrima* consumes O_2 faster than *Saccharomyces cerevisiae* and then other studied *non-Saccharomyces* species. The O_2 consumption was also evaluate in the context of a yeast mix used as industrial bioprotection (*Metschnikowia pulcherrima* and *Torulaspora delbrueckii*) in red must. These non-*Saccharomyces* yeasts were then showed to limit the growth of acetic acid bacteria, with a bioprotective effect comparable to that of the addition of sulfur dioxide. Laboratory experiment confirmed the negative impact of the non-*Saccharomyces* yeasts on *Gluconobacter oxydans* that may be related to O_2 consumption. This study sheds new lights on the use of bioprotection as an alternative to SO_2 and suggest the possibility to use O_2 consumption measurements as a new criteria for non-*Saccharomyces* strain selection in a context of bioprotection application for the wine industry.

KEYWORDS

Oenology; bioprotection; *Metschnikowia pulcherrima*; *Gluconobacter oxydans*; O_2 consumption rate; sulphur dioxide alternative

HIGHLIGHTS

- OCR is significantly different both at the inter et intra species level
- Bioprotection and cold temperatures limit the growth of acetic acid bacteria
- OCR is a new parameter for industrial bioprotection selection

31 # 1. INTRODUCTION

32 Sulfur dioxide (SO_2) is the most used additive in the winemaking process. Reducing its doses with
33 alternatives is the subject of research projects, particularly in oenology (Lisanti et al., 2019). Its multiple
34 properties, namely antimicrobial, antioxidant, antioxidasic and its low cost explain its wide use.
35 However, SO_2 in winemaking process is pointed out because it can cause intolerance in humans (Timbo
36 et al., 2004; Vally et al., 2009; Warner et al., 2000). Indeed, SO_2 has been shown to play a role in adverse
37 reactions in a small population of "sulfite-sensitive" individuals (about 1%) (Papazian, 1996). In
38 addition, the consumer's view of the agrifood products has changed as they are now more aware of
39 preservative compounds, and thus wish to limit these products in items they consume (Apaolaza et al.,
40 2017; D'Amico et al., 2016). On the basis of health problems, European regulations (Conventional
41 wines: Regulation (EC) N° 606/2009 Annex1B) reduced maximum doses authorized in winemaking by
42 50 mg/L for organic specifications: the doses must be under 100 mg/L of total SO_2 for red wines (< 2g/L
43 of sugars). Thus, searching for SO_2 alternatives has been a major challenge in oenology for the last
44 decade. Among them, chemical solutions (ascorbic acid, sorbic acid, DMDC, etc.) or physical methods
45 (filtration and thermal treatment) that can permits to reduce microbial spoilage of must or wine have
46 been proposed (Lisanti et al., 2019). An emerging alternative concerns bioprotection (Nardi, 2020). This
47 strategy is largely applied for food preservation (Leyva Salas et al., 2017; Lücke, 2000) and has more
48 recently been developed for wine (Di Gianvito et al., 2022; Morata et al., 2019); it consists in the early
49 addition of microorganisms (mainly non-*Saccharomyces* yeasts) directly to the harvest, in the press or
50 at vatting to replace the first SO_2 addition.

51 Recent studies have established the effectiveness of bioprotection in oenology, particularly related to its
52 antimicrobial properties and niche occupation during the winemaking process. The use of non-
53 *Saccharomyces* yeast in grape must, indeed allows the colonization of the environment, thus preventing
54 the development of spoilage microorganisms during the prefermentary stages, such as *Brettanomyces*
55 *bruxellensis* (Simonin et al., 2020, 2018; Windholtz et al., 2021d, 2021a). Acetic acid bacteria (AAB)
56 are among other microorganisms susceptible to alter the musts and wines quality. AAB produce acetic
57 acid, ethyl acetate leading to "vinegar" and "nail polish-remover" off-favors as well as SO_2 combining
58 products such as gluconic acid (Bartowsky and Henschke, 2008; Du Toit and Pretorius, 2000;
59 Ramachandran et al., 2006). The impact of sulfur dioxide on AAB is not clearly established (Andorrà et
60 al., 2008; Joyeux et al., 1984a) and other means to limit their impact and growth such as filtration and
61 flash pasteurization have been proposed (Lisanti et al., 2019). A recent work reported a potential action
62 of a mix of *Torulaspora delbrueckii* and *Lachancea thermotolerans* applied as a bioprotection agent on
63 AAB population levels at the start of alcoholic fermentation (Escribano-Viana et al. 2022).

64 Recently, the antioxidant potential of non-*Saccharomyces* yeasts was reported to be related to their
65 oxygen consumption (Giménez et al., 2023; Windholtz et al., 2021b). Simonin et al. (2018) showed a

66 decrease of dissolved O_2 in white must treated with bioprotection (*Torulaspora delbrueckii*).
67 *Metschnikowia pulcherrima* was also reported to reduce browning intensity of the must because it
68 consumes oxygen very efficiently and reduces its availability for the polyphenol oxidases (Giménez et
69 al., 2023).However, only few strains and species of non-*Saccharomyces* yeast have been considered
70 until now and their impact on AAB population level was so far not been evaluated.

71 The aims of this work were (i) to study the antioxidant properties and to assess the oxygen consumption
72 of non-*Saccharomyces* yeasts; (ii) to evaluate their impact on AAB as bioprotection in a context of no
73 added sulfur dioxide during red winemaking process. A large collection of non-*Saccharomyces* yeasts
74 representative of the genetic diversity of the species naturally present in grape must was considered. The
75 O_2 consumption of non-*Saccharomyces* yeasts and of a commercial bioprotection agent composed of
76 two species *Torulaspora delbrueckii* and *Metschnikowia pulcherrima* (BP) on grape must was studied
77 as well as their impact on AAB growth.

78

2. MATERIALS & METHODS

2.1. INTER AND INTRASPECIES DIVERSITY OF YEAST AND O_2 CONSUMPTION

2.1.1. Biological material

Strains from different collections and belonging to different species were selected: *Torulaspora delbrueckii* (n=7), *Saccharomyces cerevisiae* (n=8), *Hanseniaspora uvarum* (n=8), *Lachancea thermotolerans* (n=9) and *Saccharomyces uvarum* (n=4), *Metschnikowia pulcherrima* (n=11, Sipiczki, 2022), (Table S1).. From storage at -80°C, each strain was cultivated on YPD-based medium (10 g/L Yeast extract, 10 g/L Peptone, 20 g/L D-Glucose, and 25 g/L agar, pH adjusted to 4.8 with orthophosphoric acid) and then subcloned on the same medium. Species identity was validated using MALDI-TOF MS (Windholtz et al., 2021a). Since no data were available for *M. pulcherrima* species, isolates were analyzed by MALDI-TOF MS and D1/D2 sequencing analysis. A main spectra library (MSP) dendrogram was constructed using a correlation distance measure and an average linkage algorithm with the MALDI Biotyper Compass Explorer (Bruker-Daltonics).

2.1.2. Experimental design

For yeast precultures, one colony was suspended in 10 mL of pasteurized red grape juice (Jafaden, France) and grown at 25°C for 2-3 days under agitation. A sample was taken to evaluate the initial viable population level. Dead and viable cell population were estimated by flow cytometer (CytoFlex, Beckman Coulter) using propidium iodide at 3µL/mL. To study the impact of yeast on oxygen consumption, the grape juice was inoculated at 10^6 viable cells/mL with the different strains. Viable population levels were measured with the flow cytometer (CytoFlex, Beckman Coulter) at the start of experiment then at the end, after O_2 was completely consumed.

2.2. EVALUATION OF OXYGEN CONSUMPTION RATE

The O_2 consumption by yeast was studied on an industrial pasteurized organic grape juice (Jafaden, France). The commercial bioprotection preparation used was Zymaflore®Egide (ZE) (Laffort, Floirac, France), is composed of a mix of *Torulaspora delbrueckii* and *Metschnikowia pulcherrima* (50/50 w/w). Pure cultures of *Torulaspora delbrueckii* (ADY TD), *Metschnikowia pulcherrima* (ADY MP) of ZE commercial Zymaflore®Egide bioprotection (Laffort, Floirac, France) and *Saccharomyces cerevisiae* (ADY SC) with Zymaflore®X-pure (Laffort, Floirac, France) strains were also used to study their O_2 consumption capacity. Active Dry Yeast (ADY) was rehydrated according to the manufacturer's recommendations at 50mg/L corresponding to 10^6 UFC/mL. Different inoculation rates, 12.5 mg/L, 25 mg/L and 50 mg/L were tested (ADY ZE).

110 In all experiments, a negative and positive control were carried out, consisting of non-inoculated grape
111 juice or ZE (50 mg/L) inoculated juice respectively. To evaluate the O_2 consumption of dead yeast cells
112 and the impact of the preculture two treatments were considered: heat inactivated ADY ZE (80 °C, 30
113 min), ZE Inoculum at 50 mg/L (10^6 cells/mL). A yeast preculture (protocol described in section 2.1.2)
114 was established for "ZE Inoculum" treatment.

115 2.3. Monitoring dissolved O_2 and evaluation of the O_2 consumption rate (OCR)

116 Grape juice oxygenated at saturation by bubbling with air (8 ± 0.2 mg/L) and bioprotection was
117 distributed in 120 mL glass bottles (Duran® borosilicate glass equipped with oxygen sensor spot) in
118 triplicates. Grape juice saturated with O_2 without yeast addition was the negative control. Bottles were
119 sealed and incubated at 13°C with agitation, to reproduce typical conditions of a pre-fermentation
120 maceration. In order to measure dissolved oxygen, a compact 4 channel FireStingO2 oximeter
121 (Pyroscience, Bionef, France) with optics fiber and sensor spot OXSP5 (Bionef, Montreuil, France)
122 inserted inside the 250 mL glass bottle was used. The bottles were filled up to the brim with the
123 experimental grape juice, closed with GL45 with septum caps (Bromobutyl, D. Dutscher, Merignac,
124 France) and slightly shaken during measurements by a multiple-position stirring plate (Thomas
125 Scientific, New Jersey, USA). The dissolved oxygen measurements started after a 10 min equilibration
126 time and were carried out by automatic on-line measurement at intervals of 10 seconds, until the total
127 O_2 consumption was achieved.

128 From the data of dissolved O_2 over time, the O_2 consumption as function of the time (ΔO_2 between initial
129 O_2 concentration and final O_2 concentration each 10 seconds) was calculated for each modality.
130 According to Ferreira et al. (2015), a trend curve and its equation were obtained using Microsoft Excel
131 (2015) and the Oxygen Consumption Rate (OCR) expressed as mg.O_2 consumed/L/h was calculated (an
132 example is presented Figure S.3). Concerning the inter and intra species diversity on O_2 consumption
133 (2.1), OCR was expressed as mg.O_2 consumed/L/h/viable cell.

134 2.4. Impact of bioprotection on acetic acid bacteria

135 2.4.1. Experimental design at the industrial scale

136 Merlot grapes from the 2018 vintage were produced in Pomerol AOC estate (Bordeaux, south-west,
137 France). In the winery, the harvest was crushed according to standard practices and distributed in new
138 225 L french oak barrels. The experiment was carried out in duplicate. A pre-fermentation maceration
139 at 10°C was performed during 48h before inoculation with 200 mg/L of commercial *Saccharomyces*
140 *cerevisiae* (Excellence® XR) to conduct alcoholic fermentation (AF).

141 Three treatments were applied: 1) bioprotection ZE was inoculated at 50 mg/L (BP modality) directly
142 on to the grapes following the manufacturer's indications; 2) 50 mg/L of SO_2 were added at vatting (SO_2
143 modality); 3) no SO_2 nor bioprotection ZE (Without SO_2 modality) were applied. Throughout the

144 winemaking process, 10 mL samples of must were collected in sterile conditions at different stages in
145 each barrel: vatting, 24h of maceration, 48h of maceration and start AF (loss of 0.01density points).
146 Acetic acid bacteria populations were monitored by quantitative PCR (Q-PCR) using specific primers
147 (laboratory personal communication). For this analysis, cells were collected from samples after
148 centrifugation at 9000 rpm during 10 min and were rinsed twice with EDTA 50mM before being frozen
149 and conserved at -20°C until subsequent DNA extraction. For DNA extraction and Q-PCR reactions,
150 the protocol was followed according to Zott et al. (2010). Classical chemical analysis (residuals sugars,
151 total acidity, malic acid, pH, ethanol (v/v) and volatile acidity was obtained with Enology Analyzer Y15
152 (BioSystems, Spain) and a WineScan^{TM} Flex (Foss, Hillroed, Denmark) coupled to Foss Integrator 2
153 software (version 2.0.2).

154 ### 2.4.2 Laboratory scale

155 The impact of the bioprotection agent ZE on *Gluconobacter oxydans* was tested at two temperatures: 10
156 and 15 °C. From storage at -80°C, strain 08ba05(isolated fromgrape berries, CRBO collection) was
157 cultivated on a selective medium (BA) (25% (v/v) of commercial red grape juice, 5g/L of yeast extract,
158 1 mL/L of Tween 80 and 25% of agar, adjusted to pH 4.8). BA medium was supplemented with
159 antibiotics, namely pimaricine at 5 g/L and penicillin at 1.25 g/L to inhibit yeast and lactic acid bacteria
160 respectively. Species identity was validated using MALDI-TOF MS (Windholtz et al., 2021a).

161 Preculture of *Gluconobacter oxydans* was performed using the following protocol: one colony was
162 suspended in 10 mL of pasteurized red grape juice (Jafaden, France) and grown at 25°C during 1 week
163 under agitation.

164 To determine viable bacteria populations in the preculture, the epifluorescence method using
165 ChemChrome V6 (Chemunex) containing fluorescein diacetate was used. The protocol described by
166 Divol et al. (2005) was applied. 1 ml of preculture was filtered on a 0.4 µm filter (Isopore^{TM}) and then
167 placed on a pad soaked in ChemChrome V6 (Chemunex) at 1 % (v/v) in ChemSol B16 buffer
168 (Chemunex). The Pad and the filter in contact were incubated in the dark at 30 °C. Finally, living cells
169 were counted (colored in green) with an epifluorescence microscope (excitation filter 480 nm and
170 emission 515 nm).

171 Then, industrial pasteurized red grape juice (Jafaden, France) saturated with O_2 (8 ± 0.2 mg/L) was
172 inoculated with ADY bioprotection at 50 mg/L and with 10^5 viable cells/mL of *G. oxydans*. For this
173 experiment, all modalities were saturated with oxygen twice: at the initial O_2 saturation and when the
174 oxygen was totally consumed (after 26h), the red juice was re-saturated with O_2 at 8 ± 0.2 mg/L. The
175 samples were collected at three different stages to monitor *G. oxydans* population levels: at start of
176 experiment (0h, after inoculation), 26h (after O_2 consumption of the initial O_2 saturation) and at the end
177 of experiment (about 48h, time to consume the second O_2 saturation). The O_2 concentration was on-line

178 monitored throughout the experimentation by the same system previously as described (section 2.3).
179 Population levels (UFC/mL) of *G. oxydans* were estimated on BA solid media.

180

181 ## 2.5. STATISTICAL ANALYSIS

182 Statistical analysis were performed on Rstudio (RStudio Team, 2022). Results are presented as
183 scatterplots using “ggplot2” package (Wickham et al., 2016), violin diagram with « ggstatsplot »
184 package (Patil, 2021), «palmerpenguins » package (Horst et al., 2020) and « tidyverse » package
185 (Wickham and Wickham, 2017).

186 Parameters of normality and homogeneity of variances were controlled using Shapiro-Wilks and
187 Levene’s tests using “car” package (Fox and Weisberg, 2018). An analysis of variance by ANOVA
188 using “agricolae” package (Mendiburu Delgado, 2009) or a Student test were used to determine
189 significant differences. The Tukey HSD test was used to classify significant modalities (if >2
190 modalities). If parameters were not respected, a Kruskall-wallis test weas applied. Also, a Dunnetts test
191 was used to compare modalities with a defined control. For this test, « DescTools » package (Signorell
192 et al., 2019), « dplyr » package (Wickham et al., 2020) and « multcomp » package (Hothorn et al., 2016)
193 were required.

194

3. RESULTS

3.1. YEAST INTER/INTRA SPECIES VARIABILITY REGARDING O_2 CONSUMPTION

In order to evaluate the inter/intra species variability of the O_2 consumption rate in grape juice, a collection of 47 strains belonging to 6 species (*Torulaspora delbrueckii*, *Saccharomyces cerevisiae*, *Hanseniaspora uvarum*, *Lachancea thermotolerans, Saccharomyces uvarum* and *M. pulcherrima*) was gathered (Table S1). Species were chosen because they are frequently reported to be associated with grape juice and proposed as commercial yeast for bioprotection (Nardi, 2020). For each species, strains were selected to represent the intra-species genetic diversity as previously reported in the literature (Albertin et al., 2016, 2014; Hranilovic et al., 2018; Marullo et al., 2020). Since no data were available on *M. pulcherrima* intraspecies genetic diversity, we used two complementary methods (MALDI-TOF MS and D1/D2 sequences analysis to construct dendrograms (Figure S.1 and S.2) to situate our isolates in the *pulcherrima* clade (Sipiczki 2022). On the D1/D2 dendrogram tree, isolates gathered in the same branch, except L0674.

Violin representations for Oxygen Consumption Rate (OCR, expressed in mg/L of O_2 consumed by viable cell and by hour) were generated for each species (Figure 1). Experiment times varied between 2.5 h and 7.5 h and the OCR mean was of 1.47 x 10^{-6} ± 0.5 x 10^{-6} mg O_2 consumed/L/h/viable cell. *M. pulcherrima* was the most efficient species to consume O_2 with a mean OCR of 1.87 x 10^{-6} ± 0.31 x 10^{-6} mg O_2 consumed/L/h/viable cell. Conversely, *Saccharomyces cerevisiae* and *Saccharomyces uvarum* were the least efficient species with a mean OCR of 1.01 x 10^{-6} ± 0.19 x 10^{-6} and 1.20 x 10^{-6} ± 0.28 x 10^{-6}, respectively. Intermediate behaviors were observed for *H. uvarum* (1.47 x 10^{-6} ± 0.42 x 10^{-6}), *L. thermotolerans* (1.49 x 10^{-6} ± 0.65 x 10^{-6}) and *T. delbrueckii* (1.48 x 10^{-6} ± 0.44 x 10^{-6}). Furthermore, a high OCR variability at the intra-species level could be highlighted, especially for *L. thermotolerans* (ranging from 0.92 x 10^{-6} ± 0.18 x 10^{-6} to 2.69 x 10^{-6} ± 0.09 x 10^{-6}) and for *H.* u*varum* (ranging from 0.98 x 10^{-6} ± 0.14 x 10^{-6} to 2.27 x 10^{-6} ± 0.22 x 10^{-6}). No correlation was observed in between the initial oxygen consumption rate (mg.O_2 consumed/L/h) values and the population levels during that time.

The O_2 consumption in the must by bioprotection can be a major advantage in the winemaking process. Therefore, the non-*Saccharomyces* mix behavior, especially in an industrial context, was then studied more in details.

3.2. EVALUATION OF O_2 CONSUMPTION BY A MIX OF NON-*SACCHAROMYCES* AS BIOPROTECTION

Two strains of *M. pulcherrima.* and *T. delbrueckii*, already available as a commercial bioprotection agent, were chosen to more deeply study their O_2 consumption properties. The objective of this part of

228 the study was to determine whether the application formulation had an impact on O_2 consumption,
229 depending on the species, alone or in combination.
230 Different experiments were considered related to yeast inoculation rate and the use of mixed or pure
231 cultures. The O_2 consumption rates (OCR express as mg.O_2 consumed/L/h) of the different treatments
232 are presented in Figure 2. For each experiment, a negative and positive control were carried out
233 consisting in grape juice with no addition of yeast and addition of ZE, respectively. The mean OCR of
234 the negative control (n=5) was of 0.087 mg/L/h ± 0.02 and the positive control (ADYZE 50 mg/L)
235 generated an OCR of 1.39 ± 0.06 mg/L/h (n=5).
236 The OCR of the heat inactivated ADYZE was not significantly different from the negative control
237 (Dunnett test), suggesting that yeast O_2 consumption is linked with yeast viability. OCRs of directly
238 applied ADY and after preculture did not differ (Dunnett test), demonstrating that the ADY formulation
239 had no impact on O_2 consumption. Figure 2 shows that the OCR of both strains increases with the
240 inoculation rate, with 0.36 ± 0.09, 0.75 ± 0.09 and 1.39 ± 0.06 mg/L/h O_2 for 10, 25 and 50 mg/L of ZE,
241 respectively. The OCR obtained from commercial ADY of different yeast species (*T. delbrueckii* (ADY
242 TD), *M. pulcherrima* (ADY MP) and *Saccharomyces cerevisiae* (ADY SC)) confirmed the results
243 previously obtained. Indeed, *M. pulcherrima* was the most efficient strain to consume O_2 ($2.54 \times 10^{-6} \pm$
244 0.59×10^{-6}) and *S. cerevisiae* the less effective ($0.42 \times 10^{-6} \pm 0.12 \times 10^{-6}$). Furthermore, the O_2
245 consumption rate by *T. delbrueckii* is not significantly different (Dunnett test) from the mix of *M.*
246 *pulcherrima* and *T. delbrueckii* (ZE). Interestingly, the ZE mix consumed significantly less O_2 than the
247 *M. pulcherrima* pure culture.
248 These results confirmed the interest of O_2 consumption by non-*Saccharomyce*s yeasts in grape juice.
249 This capacity could be an additional selection criteria for bioprotection products in winemaking process.
250 A final aspect was thus addressed, concerning the impact of bioprotection yeast on acetic acid bacteria.
251

252 ### 3.3. IMPACT OF NON-*SACCHAROMYCES MIX* ON ACETIC ACID BACTERIA

253 Acetic acid bacteria (AAB) are strictly aerobic microorganisms and oxygen is an essential growth factor
254 (Bartowsky and Henschke, 2008).The risk of spoilage by AAB during wine production can be controlled
255 by niche occupation and by eliminating or limiting oxygen. Our previous results showed that the
256 bioprotection consumed dissolved O_2 in the must. Thus, the aim of this study was to test the hypothesis
257 that the use of non-*Saccharomyces* yeasts as bioprotection could negatively impact AAB population
258 level and growth and may be related to O_2 consumption. The impact of bioprotection ZE was tested
259 through an experiment at the industrial scale conducted in a Pomerol wine estate. Indigenous AAB
260 population was monitored during the first stages of the winemaking process, by quantitative PCR
261 method. Three treatments were implemented (SO_2, without SO_2 and BP) on Merlot must (Figure 3) in
262 barrels of 225 L. In this experiment, bioprotection implantation was verified by Maldi-TOF MS analysis
263 on colonies (data not shown). Analysis of wine after alcoholic fermentation was presented in Tableau

S.3. No significant differences were noticed for the classical enological parameters. A significant difference could be observed for AAB population at the end of the prefermentary maceration. Without SO_2, the AAB population reached 5.10^7 UFC/mL (4 log increase) while with SO_2 reached only 3.10^4 UFC/mL (increase 1 log increase). Interestingly, the AAB population remained stable with BP (5.10^3-10^4UFC/mL) and even decreased at Start AF stage.

G. oxydans is the most abundant species among acetic acid bacteria present in grape must (Joyeux et al., 1984a). At laboratory scale, the impact of bioprotection ZE on *G. oxydans* was tested in standardized grape juice, at two temperatures (10 and 15 °C) in presence of O_2 at saturation. The OCR obtained are presented in Figure 4. As expected, OCR are significantly higher in the presence of bioprotection ZE. At 10°C, the OCR was of 0.822 ± 0.018 mg/L/h and 2.432 ± 0.068 mg/L/h, for 0h to 26h and 26h to 48h, respectively when bioprotection ZE was added. The OCR was of 0.010 ± 0.023 mg/L/h and 0.060 ± 0.0035 mg/L/h for *G. oxydans* alone. The OCRs were significantly higher at 15°C than at 10°C and significantly increased after the second addition of O_2 at saturation for all treatments. The presence of bioprotection ZE reduced significantly the growth of *G. oxydans* after 48h at 10°C and 15°C (significant at 0.05). The combination of bioprotection ZE addition and a low temperature (10°C) led to a decrease of the *G. oxydans* population from 24h by one log, to achieve 10^4 UFC/mL at the end of the experiment.

4. Discussion

4.1. Evaluation of O_2 consumption by *non-Saccharomyces* yeast

In this study, we aimed at evaluating O_2 consumption by yeast in a context of bioprotection utilization during the prefermentary winemaking stages. Must can be exposed to O_2 during crushing, pressing, or pumping (Schneider, 1998; Toit et al., 2006). Moreover, in the without SO_2 conditions, polyphenol oxidases are not inhibited. So, protection from oxidation during prefermentary stages in order to limit the O_2 dissolved concentrations is a key issue especially when no SO_2 is used. In the present study, tests were implemented to monitor the dissolved oxygen and the ability of different species of non-*Saccharomyces* yeast to consume it. We aim to define an OCR value associated with different bioprotective yeast. Significant differences appeared both at the inter and intraspecies levels; among the six species tested, *S. uvarum* and *S. cerevisiae* had the lowest OCR, whereas *M. pulcherrima* was most efficient species. The O_2 consumption ability in grape juice was showed to be related to yeast cell viability, but with no correlation with cell multiplication. Active Dry Yeast (ADY) are produced at the industrial scale by aerobic metabolism in the presence of low sugar concentrations, that could impact the ADY yeast cell metabolism in favor of the respiration one. Our data showed that the O_2 consumption was not related to the production under an ADY form, with similar levels obtained with direct inoculation of ADY after rehydration or with a pre-culture. For winemakers, bioprotection by adding mix between *M. pulcherrima.* and *T. delbrueckii* is normally applied at 50 mg/L directly on the grape or

298 after vatting stage following manufacturer protocol. Application of the mix at a higher dosage (over 50
299 mg/L) could limit *Saccharomyces cerevisiae* implantation during the alcoholic fermentation, as
300 demonstrated by (Windholtz et al., 2021c), resulting in a higher volatile acidity content in the final wine
301 and stuck alcoholic fermentation. The consumption of O_2 by the mix was higher at 50 mg/L than at 10
302 or 25 mg/L. Nevertheless, the application rate could be increased in the case of a harvest at an advanced
303 maturity, to allow better occupation space in must (Windholtz et al., 2021d). Overall, our results support
304 the importance of adjusting the dose of the yeast preparation in a context of bioprotection application.

305 In eukaryotic cells, oxygen is metabolized to perform both catabolic and anabolic functions. The
306 classical mitochondrial respiratory chain through oxidative phosphorylation, leads to ATP synthesis. In
307 grape must, yeast species are mainly facultative anaerobic, i.e. they also have capacity to metabolize
308 glucose by fermentation. Yeasts can be subdivided into two groups, according to their capacity to carry
309 out aerobic fermentation based on the Crabtree effect (Deken, 1966 ; Gancedo and Serrano, 1989). This
310 is linked to a catabolic repression by glucose that inhibits respiration, favoring alcoholic fermentation.
311 *Saccharomyces cerevisiae* is known to be strongly Crabtree positive (Swanson and Clifton, 1948). When
312 the glucose concentration is higher than 1g/L, a catabolic repression of glucose occurs thus inhibiting
313 respiration to the benefit of fermentation (Barnett and Entian, 2005). *Torulaspora delbrueckii* and
314 *Lachancea thermotolerans* are also reported as Crabtree positive (Gonzalez et al., 2013). *Hanseniaspora*
315 *uvarum* and *Metschnikowia pulcherrima* are considered as Crabtree negative (Mencher et al., 2021;
316 Venturin et al., 1994) with a higher respiration capacity compared to *Saccharomyces cerevisiae* and an
317 enhanced biomass production (Schnierda et al., 2014). Aerobic species show approximately three times
318 higher respiratory fluxes of the tricarboxylic acid cycle (TCA cycle) than *Saccharomyces cerevisiae*. In
319 our experimental conditions, the hypothesis that Crabtree negative species consume higher amounts of
320 dissolved O_2 in grape must could not explain the differences among species; *Hanseniaspora uvarum*, as
321 Crabtree negative, consumes equivalent amount of O_2 than *Torulaspora delbrueckii* and *Lachancea*
322 *thermotolerans*, both Crabtree positive species.

323 In addition to the Crabtree effect and thus to respiration, different metabolic pathways use molecular O_2
324 (Rosenfeld and Beauvoit, 2003) such as lipids, amino acids, vitamins, iron or ubiquinone metabolisms.
325 Yeast consume O_2 for growth and lipid synthesis (sterols and unsaturated fatty acids for membrane
326 integrity) (Salmon, 2006). *Metschnikowia pulcherrima* species is also studied in a completely different
327 context, as an oleaginous yeasts. These yeast produce microbial lipids that can reach more than 20 % of
328 their dry cell weight as lipids (Thorpe and Ratledge, 1972). They have been studied as a promising
329 alternative to vegetal oils, for direct replacement in food applications or biodiesel production (Sitepu et
330 al., 2014). Lipid biosynthesis by such yeast and their accumulation is initiated with a limited excess
331 carbon and nutrient source (usually nitrogen) and they exhibit an ability to consistently supply acetyl-
332 CoA and NADPH (Sutanto et al., 2018). As lipid synthesis is also correlated with O_2 consumption (Nioi

333 et al., 2022), this particular metabolic pathways in *Metschnikowia pulcherrima* could explain its higher
334 O_2 consumption capacity compared to other species.

335 Finally, yeast metabolic interactions can take place, resulting in a modification of the aerobic respiration
336 by microorganisms. Mencher et al. (2021) showed an impact of co-inoculation with *Metschnikowia*
337 *pulcherrima* on the *Saccharomyces cerevisiae* metabolism during alcoholic fermentation. Glycolysis
338 was increased while the TCA cycle was decreased. They also observed that *Metschnikowia pulcherrima*
339 had a higher respiration capacity compared to *Saccharomyces cerevisiae* and was able to deplete O_2
340 from the medium at a higher rate, which is congruent with our results. Our data shows that the ZE mix
341 consumed significantly less O_2 than the *M. pulcherrima* pure culture, for a given inoculation rate (50
342 mg/L). This result is difficult to interpret without precise population quantification of each species in
343 the mix. Indeed, a negative interaction between the two species in the ZE mix may lead to a higher
344 percentage of *T. delbrueckii* at population level thus explaining that the OCR is lower than with *M.*
345 *pulcherrima* alone. Indeed, the mix of this two species could be an advantage: both species occupies the
346 niche (Windholtz et al., 2021a,d), *Metschnikowia pulcherrima* is efficient for O_2 consumption whereas
347 *Torulaspora delbrueckii* enhance the wine fruitiness in without SO_2 context (Windholtz et al., 2021c).

348 Aside from the use of sulfur dioxide, very few alternatives with antioxidant properties are proposed for
349 the winemaking process. Ascorbic acid is an alternative but should always be associated with low doses
350 of SO_2 (Bradshaw et al., 2004); oenological tannins (González-Centeno et al., 2012; Magalhães et al.,
351 2014; Pascual et al., 2017; Vignault et al., 2020); yeast derivatives (Inactive Dry Yeast Preparations)
352 (Bahut et al., 2020; Nioi et al., 2022) and inactivated dry yeasts rich in glutathione (Gimenez et al.,
353 2023) can also be considered. Recently, preliminary studies were carried out on the bioprotection by
354 using non-*Saccharomyces* yeasts as an antioxidant potential. The use of non-*Saccharomyces* resulted in
355 a consumption of O_2 dissolved in must, reducing its browning while maintaining GSH concentrations
356 in white wines (Binati et al., 2021; Giménez et al., 2023; Windholtz et al., 2021b). Our study shows that
357 the high O_2 consumption capacity is a trait associated with *Metschnikowia pulcherrima* and some strains
358 of *Lachancea thermotolerans* that could be relevant candidates in a selection for bioprotection
359 application.

360 4.2. NEGATIVE IMPACT OF NON-*SACCHAROMYCES* ON ACETIC ACID BACTERIA

361 In grape must, the presence of acetic acid bacteria (AAB) is likely to produce ethyl acetate and acetic
362 acid (Drysdale and Fleet, 1989; Du Toit and Pretorius, 2000). These microorganisms are obligate aerobic
363 (De Ley, 1984) able of oxidizing ethanol into acetic acid (Joyeux et al., 1984a).

364 Three genera are mainly associated with must and wine spoilage: *Acetobacter*, *Gluconobacter* and
365 *Gluconacetobacter* (Bartowsky and Henschke, 2008). *Gluconobacter oxydans* is the major species in
366 the must although, in the case of grapes with grey rot, *Acetobacter aceti* can also be found at high

367 population levels (Du Toit and Lambrechts, 2002; Joyeux et al., 1984b). The effectiveness of SO_2 on
368 these spoilages is not clearly established in the literature. Moreover, in the case of botrytised grapes,
369 AAB produce compounds that combine SO_2, thus reducing its effectiveness (Barbe et al., 2001; Joyeux
370 et al., 1984b).

371 Until now, the antimicrobial effect of bioprotection using *Metschnikowia pulcherrima* has been reported
372 on *Hanseniaspora uvarum* (Albertin et al., 2016; Canonico et al., 2023; Johnson et al., 2020; Jolly et al.,
373 2014). Simonin et al. (2020) also showed a potential impact on *Brettanomyces bruxellensis* during
374 prefermentation stages. This species occupies the must environment without affecting its organoleptic
375 properties and with very low fermentative activity (Morata et al., 2019; Windholtz et al., 2021a).
376 Bioprotection composed of two non-*Saccharomyces* species (*Torulaspora delbrueckii* and
377 *Metschnikowia pulcherrima*) also showed an antimicrobial activity by occupying the niche thus
378 reducing the relative abundance of *Hanseniaspora uvarum* and filamentous fungi during prefermentary
379 stages (Johnson et al., 2020 ; Windholtz et al., 2021c; Windholtz et al., 2021a). Escribano-Viana et al.
380 (2022) showed an impact of *Torulaspora delbrueckii* and *Lachancea thermotolerans* as bioprotective
381 agents on AAB development during the first stages of the winemaking process. In the present study, we
382 confirmed that the presence of *Torulaspora delbrueckii* and *Metschnikowia pulcherrima* negatively
383 impacts AAB population levels during prefermentary stages. At the winery scale, this bioprotective
384 effect was comparable and even greater than sulfur dioxide addition as it was reported by Escribano-
385 Viana et al. (2022) using *Torulaspora delbrueckii* and *Lachancea thermotolerans*. We suggest that the
386 negative interactions between yeast and obligate aerobic bacteria that could be partially mediated by the
387 O_2 consumption in must by BP. The negative interaction between non-*Saccharomyces* yeast and AAB
388 is reinforced at low temperature (10°C) even if the colder the temperature, the more O_2 dissolves in the
389 medium (Schlaepfer, 1949).

390 # 5. CONCLUSION

391 In this study, the O_2 consumption capacity by non-*Saccharomyces* was characterized and quantified by
392 OCR evaluation, both at interspecies and intraspecies levels. *M. pulcherrima* was found to be the most
393 efficient O_2 consumer. The use of *Torulaspora delbrueckii* and *Metschnikowia pulcherrima* negatively
394 impacts AAB population levels during prefermentary stage and this bioprotective effect was comparable
395 and even greater than sulfur dioxide addition. Further studies would be needed to study the interactions,
396 competition and space occupation phenomena between non-*Saccharomyces* yeast used as bioprotection
397 and AAB. Finally, this study highlights the possibility to use the O_2 consumption as a new criteria for
398 non-*Saccharomyces* strain selection in a context of bioprotection application for the wine industry.

399 # CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

400 **Sara Windholtz**: Conceptualization, Investigation, formal analysis, Visualisation, Writing-original
401 draft; **Claudia Nioi**: Conceptualization, Methodology, supervision, Writing – review & editing; **Joana**
402 **Coulon**: Funding acquisition, Supervision, Ressources, Writing – review & editing; **Isabelle Masneuf-**
403 **Pomarède**: conceptualization, Funding acquisition, Supervision, Writing – review & editing.

404 # DECLARATION OF COMPETING INTEREST

405 The authors declare that they have no known competing financial interests or personal relationships
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411 # REFERENCES

412 Albertin, W., Chasseriaud, L., Comte, G., Panfili, A., Delcamp, A., Salin, F., Marullo, P., Bely, M., 2014.
413 Winemaking and bioprocesses strongly shaped the genetic diversity of the ubiquitous yeast
414 *Torulaspora delbrueckii*. PLoS One 9, e94246. https://doi.org/10.1371/journal.pone.0094246
415 Albertin, W., Chernova, M., Durrens, P., Guichoux, E., Sherman, D.J., Masneuf-Pomarede, I., Marullo,
416 P., 2018. Many interspecific chromosomal introgressions are highly prevalent in Holarctic
417 *Saccharomyces uvarum* strains found in human-related fermentations. Yeast 35, 141–156.
418 https://doi.org/10.1002/yea.3248
419 Albertin, W., Setati, M.E., Miot-Sertier, C., Mostert, T.T., Colonna-Ceccaldi, B., Coulon, J., Girard, P.,
420 Moine, V., Pillet, M., Salin, F., Bely, M., Divol, B., Masneuf-Pomarede, I., 2016. *Hanseniaspora*
421 *uvarum* from Winemaking Environments Show Spatial and Temporal Genetic Clustering.
422 Front. Microbiol. 6, 1569. https://doi.org/10.3389/fmicb.2015.01569
423 Andorrà, I., Landi, S., Mas, A., Guillamón, J.M., Esteve-Zarzoso, B., 2008. Effect of oenological
424 practices on microbial populations using culture-independent techniques. Food Microbiol.
425 25, 849–856. https://doi.org/10.1016/j.fm.2008.05.005
426 Apaolaza, V., Hartmann, P., Echebarria, C., Barrutia, J.M., 2017. Organic label's halo effect on sensory
427 and hedonic experience of wine: A pilot study. J. Sens. Stud. 32, e12243.
428 https://doi.org/10.1111/joss.12243
429 Bahut, F., Romanet, R., Sieczkowski, N., Schmitt-Kopplin, P., Nikolantonaki, M., Gougeon, R.D., 2020.
430 Antioxidant activity from inactivated yeast: Expanding knowledge beyond the glutathione-
431 related oxidative stability of wine. Food Chem. 325, 126941.
432 https://doi.org/10.1016/j.foodchem.2020.126941

433 Barbe, J.-C., De Revel, G., Joyeux, A., Bertrand, A., Lonvaud-Funel, A., 2001. Role of botrytized grape
434 micro-organisms in SO_2 binding phenomena. J. Appl. Microbiol. 90, 34–42.
435 https://doi.org/10.1046/j.1365-2672.2001.01200.x
436 Barnett, J.A., Entian, K.-D., 2005. A history of research on yeasts 9: regulation of sugar metabolism1.
437 Yeast 22, 835–894. https://doi.org/10.1002/yea.1249
438 Bartowsky, E.J., Henschke, P.A., 2008. Acetic acid bacteria spoilage of bottled red wine—A review.
439 Int. J. Food Microbiol., 125, 1, 60-70.
440 https://doi.org/10.1016/j.ijfoodmicro.2007.10.016
441 Binati, R. l., Lemos Junior, W. j. f., Torriani, S., 2021. Contribution of non-*Saccharomyces* yeasts to
442 increase glutathione concentration in wine. Aust. J. Grape Wine Res. 27, 290–294.
443 https://doi.org/10.1111/ajgw.12473
444 Bradshaw, M.P., Scollary, G.R., Prenzler, P.D., 2004. Examination of the sulfur dioxide–ascorbic acid
445 anti-oxidant system in a model white wine matrix. J. Sci. Food Agric. 84, 318–324.
446 https://doi.org/10.1002/jsfa.1652
447 Canonico, L., Agarbati, A., Galli, E., Comitini, F., Ciani, M., 2023. *Metschnikowia pulcherrima* as
448 biocontrol agent and wine aroma enhancer in combination with a native *Saccharomyces*
449 *cerevisiae*. LWT 181, 114758.
450 https://doi.org/10.1016/j.lwt.2023.114758
451 D'Amico, M., Di Vita, G., Monaco, L., 2016. Exploring environmental consciousness and consumer
452 preferences for organic wines without sulfites. J. Clean. Prod. 120, 64–71.
453 https://doi.org/10.1016/j.jclepro.2016.02.014
454 De Ley, J., 1984. Genus Acetobacter. Bergey's manual of systematic bacteriology. 1, 268–274.
455 Di Gianvito, P., Englezos, V., Rantsiou, K., Cocolin, L., 2022. Bioprotection strategies in winemaking.
456 Int. J. Food Microbiol. 364, 109532.
457 https://doi.org/10.1016/j.ijfoodmicro.2022.109532
458 Divol, B., Strehaiano, P., Lonvaud-Funel, A., 2005. Effectiveness of dimethyldicarbonate to stop
459 alcoholic fermentation in wine. Food Microbiol. 22, 169–178.
460 http://dx.doi.org/10.1016/j.fm.2004.07.003
461 Drysdale, G.S., Fleet, G.H., 1989. The growth and survival of acetic acid bacteria In wines at different
462 concentrations of oxygen. Am. J. Enol. Vitic. 40, 99–105.
463 https://doi.org/10.5344/ajev.1989.40.2.99
464 Du Toit, M., Pretorius, I.S., 2000. Microbial spoilage and preservation of wine: using weapons for
465 nature's own arsenal. South African J. Enol. Vitic. 21, 1, 74-96.
466 https://doi.org/10.21548/21-1-3559
467 Du Toit, W.J., Lambrechts, M.G., 2002. The enumeration and identification of acetic acid bacteria
468 from South African red wine fermentations. Int. J. Food Microbiol. 74, 57–64.
469 Escribano-Viana, R., González-Arenzana, L., Garijo, P., Fernández, L., López, R., Santamaría, P.,
470 Gutiérrez, A.R., 2022. Bioprotective Effect of a *Torulaspora delbrueckii*/*Lachancea*
471 *thermotolerans*-Mixed Inoculum in Red Winemaking. Fermentation, 8, 337.
472 https://doi.org/10.3390/fermentation8070337
473 Ferreira, V., Carrascon, V., Bueno, M., Ugliano, M., Fernandez-Zurbano, P., 2015. Oxygen
474 consumption by red wines. Part I: consumption rates, relationship with chemical
475 composition, and role of SO_2. J. Agric. Food Chem. 63, 10928–10937.
476 https://doi.org/10.1021/acs.jafc.5b02988
477 Fox, J., Weisberg, S., 2018. An R companion to applied regression. Sage publications.
478 Freel, K.C., Charron, G., Leducq, J.-B., Landry, C.R., Schacherer, J., 2015. *Lachancea quebecensis* sp.
479 nov., a yeast species consistently isolated from tree bark in the Canadian province of Québec.
480 Int. J. Syst. Evol. Microbiol. 65, 3392–3399.
481 Gancedo, C. and Serrano, R. (1989) Energy-Yielding Metabolism. In Rose, A.H. and Harrison, J.S., Eds.,
482 The Yeasts, 2nd Edition, Vol. 3, Academic Press, London, 205-259.
483 Giménez, P., Just-Borras, A., Pons, P., Gombau, J., Heras, J.M., Sieczkowski, N., Canals, J.M., Zamora,
484 F., 2023. Biotechnological tools for reducing the use of sulfur dioxide in white grape must and

485 preventing enzymatic browning: glutathione; inactivated dry yeasts rich in glutathione; and
486 bioprotection with *Metschnikowia pulcherrima*. Eur. Food Res. Technol. 249, 6, 1491-1501.
487 https://doi.org/10.1007/s00217-023-04229-6
488 Gonzalez, R., Quirós, M., Morales, P., 2013. Yeast respiration of sugars by non-*Saccharomyces* yeast
489 species: a promising and barely explored approach to lowering alcohol content of wines.
490 Trends Food Sci. Technol. 29, 55–61.
491 https://doi.org/10.1016/j.tifs.2012.06.015
492 González-Centeno, M.R., Jourdes, M., Femenia, A., Simal, S., Rosselló, C., Teissedre, P.-L., 2012.
493 Proanthocyanidin composition and antioxidant potential of the stem winemaking byproducts
494 from 10 different grape varieties (*Vitis vinifera* L.). J. Agric. Food Chem. 60, 11850–11858.
495 https://doi.org/10.1021/jf303047k
496 Horst, A.M., Hill, A.P., Gorman, K.B., 2020. Palmerpenguins: Palmer Archipelago (Antarctica) penguin
497 data. R package version 0.1. 0.
498 Hothorn, T., Bretz, F., Westfall, P., Heiberger, R.M., Schuetzenmeister, A., Scheibe, S., Hothorn, M.T.,
499 2016. Package ‘multcomp.’ Simultaneous inference in general parametric models. Project for
500 Statistical Computing, Vienna, Austria.
501 Hranilovic, A., Bely, M., Masneuf-Pomarede, I., Jiranek, V., Albertin, W., 2017. The evolution of
502 *Lachancea thermotolerans* is driven by geographical determination, anthropisation and flux
503 between different ecosystems. PLoS One 12, e0184652.
504 Hranilovic, A., Gambetta, J.M., Schmidtke, L., Boss, P.K., Grbin, P.R., Masneuf-Pomarede, I., Bely, M.,
505 Albertin, W., Jiranek, V., 2018. Oenological traits of *Lachancea thermotolerans* show signs of
506 domestication and allopatric differentiation. Sci. Rep. 8, 14812.
507 https://doi.org/10.1038/s41598-018-33105-7
508 Johnson, J., Fu, M., Qian, M., Curtin, C., Osborne, J.P., 2020. Influence of Select Non-*Saccharomyces*
509 Yeast on *Hanseniaspora uvarum* Growth during Prefermentation Cold Maceration. Am. J.
510 Enol. Vitic. 71, 4, 278-287.
511 https://doi.org/10.5344/ajev.2020.20004
512 Jolly, N.P., Varela, C., Pretorius, I.S., 2014. Not your ordinary yeast: non-*Saccharomyces* yeasts in
513 wine production uncovered. FEMS Yeast Res. 14, 215–237.
514 https://doi.org/10.1111/1567-1364.12111
515 Joyeux, A., Lafon-Lafourcade, S., Ribéreau-Gayon, P., 1984a. Metabolism of acetic acid bacteria in
516 grape must. Consequences on alcoholic and malolactic fermentation. Sciences des Aliments
517 4, 247–255.
518 Joyeux, A., Lafon-Lafourcade, S., Ribéreau-Gayon, P., 1984b. Evolution of Acetic Acid Bacteria During
519 Fermentation and Storage of Wine. Appl. Environ. Microbiol. 48, 153–156.
520 https://doi.org/10.1128/aem.48.1.153-156.1984
521 Leyva Salas, M., Mounier, J., Valence, F., Coton, M., Thierry, A., Coton, E., 2017. Antifungal Microbial
522 Agents for Food Biopreservation—A Review. Microorganisms 5, 37.
523 https://doi.org/10.3390/microorganisms5030037
524 Lisanti, M.T., Blaiotta, G., Nioi, C., Moio, L., 2019. Alternative methods to SO_2 for microbiological
525 stabilization of wine. Compr. Rev. Food Sci. Food Saf. 18, 455–479.
526 https://doi.org/10.1111/1541-4337.12422
527 Lücke, F.-K., 2000. Utilization of microbes to process and preserve meat. Meat sci. 56, 105–115.
528 https://doi.org/10.1016/S0309-1740(00)00029-2
529 Magalhães, L.M., Ramos, I.I., Reis, S., Segundo, M.A., 2014. Antioxidant profile of commercial
530 oenological tannins determined by multiple chemical assays. Aust. J. Grape Wine Res. 20, 72–
531 79.
532 https://doi.org/10.1111/ajgw.12058
533 Marullo, P., Claisse, O., Raymond Eder, M.L., Börlin, M., Feghali, N., Bernard, M., Legras, J.-L.,
534 Albertin, W., Rosa, A.L., Masneuf-Pomarede, I., 2020. *SSU1* Checkup, a rapid tool for
535 detecting chromosomal rearrangements related to the *SSU1* promoter in *Saccharomyces*

536 *cerevisiae*: an ecological and technological study on wine yeast. Front. Microbiol. 11, 1331.
537 https://doi.org/10.3389/fmicb.2020.01331
538 Mencher, A., Morales, P., Curiel, J.A., Gonzalez, R., Tronchoni, J., 2021. *Metschnikowia pulcherrima*
539 represses aerobic respiration in *Saccharomyces cerevisiae* suggesting a direct response to co-
540 cultivation. Food Microbiol. 94, 103670.
541 https://doi.org/10.1016/j.fm.2020.103670
542 Mendiburu Delgado, F. de, 2009. Una herramienta de análisis estadístico para la investigación
543 agrícola. Universidad Nacional de Ingeniería, Lima (Perú).
544 Morata, A., Loira, I., Escott, C., del Fresno, J.M., Bañuelos, M.A., Suárez-Lepe, J.A., 2019. Applications
545 of *Metschnikowia pulcherrima* in Wine Biotechnology. Fermentation 5, 63.
546 https://doi.org/10.3390/fermentation5030063
547 Nardi, T., 2020. Microbial resources as a tool for enhancing sustainability in winemaking.
548 Microorganisms 8, 507.
549 https://doi.org/10.3390%2Fmicroorganisms8040507
550 Nioi, C., Lisanti, M.T., Meunier, F., Redon, P., Massot, A., Moine, V., 2022. Antioxidant activity of
551 yeast derivatives: Evaluation of their application to enhance the oxidative stability of white
552 wine. LWT 171, 114116.
553 https://doi.org/10.1016/j.lwt.2022.114116
554 Papazian, R., 1996. Sulfites, safe for most, dangerous for some. Dept. of Health and Human Services,
555 Public Health Service, Food and Drug Administration.
556 Pascual, O., Vignault, A., Gombau, J., Navarro, M., Gómez-Alonso, S., García-Romero, E., Canals, J.M.,
557 Hermosín-Gutíerrez, I., Teissedre, P.-L., Zamora, F., 2017. Oxygen consumption rates by
558 different oenological tannins in a model wine solution. Food Chem. 234, 26–32.
559 https://doi.org/10.1016/j.foodchem.2017.04.148
560 Patil, I., 2021. Visualizations with statistical details: The “ggstatsplot” approach. JOSS 6, 3167.
561 https://doi.org/10.21105/joss.03167
562 Ramachandran, S., Fontanille, P., Pandey, A., Larroche, C., 2006. Gluconic acid: properties,
563 applications and microbial production. Food Technol. Biotechnol. 44,2.
564 Rosenfeld, E., Beauvoit, B., 2003. Role of the non-respiratory pathways in the utilization of molecular
565 oxygen by *Saccharomyces cerevisiae*. Yeast 20, 1115–1144.
566 RStudio Team, 2022. RStudio: integrated development environment for R. RStudio, PBC, Boston, MA.
567 Salmon, J.-M., 2006. Interactions between yeast, oxygen and polyphenols during alcoholic
568 fermentations: practical implications. LWT, European Symposium on Apple Processing 39,
569 959–965.
570 https://doi.org/10.1016/j.lwt.2005.11.005
571 Schlaepfer, P., 1949. Untersuchungen uber das maximale Sauerstoffaufnahmevermogen organischer
572 Flussigkeiten und die jodometrische Bestimmung des aufgenommenen Sauerstoffes.
573 Schweiz. Arch. Angew. Wiss. Tech. 15, 299–307.
574 Schneider, V., 1998. Must Hyperoxidation: A Review. Am J Enol Vitic. 49, 65–73.
575 Schnierda, T., Bauer, F.F., Divol, B., Van Rensburg, E., Görgens, J.F., 2014. Optimization of carbon and
576 nitrogen medium components for biomass production using non-*Saccharomyces* wine yeasts.
577 Lett. Appl. Microbiol. 58, 478–485.
578 https://doi.org/10.1111/lam.12217
579 Signorell, A., Aho, K., Alfons, A., Anderegg, N., Aragon, T., Arppe, A., Baddeley, A., Barton, K., Bolker,
580 B., Borchers, H.W., 2019. DescTools: Tools for descriptive statistics. R package version 0.99
581 28, 17.
582 Simonin, S., Alexandre, H., Nikolantonaki, M., Coelho, C., Tourdot-Maréchal, R., 2018. Inoculation of
583 *Torulaspora delbrueckii* as a bio-protection agent in winemaking. Food Res. Int. 107, 451–
584 461.
585 https://doi.org/10.1016/j.foodres.2018.02.034
586 Simonin, S., Roullier-Gall, C., Ballester, J., Schmitt-Kopplin, P., Quintanilla-Casas, B., Vichi, S., Peyron,
587 D., Alexandre, H., Tourdot-Maréchal, R., 2020. Bio-Protection as an alternative to sulphites:

588 impact on chemical and microbial characteristics of red wines. Front. Microbiol. 11, 1308.
589 https://doi.org/10.3389/fmicb.2020.01308
590 Sipiczki, M., 2022. Taxonomic Revision of the *pulcherrima* Clade of *Metschnikowia* (Fungi): Merger of
591 Species. Taxonomy 2, 107–123.
592 https://doi.org/10.3390/taxonomy2010009
593 Sitepu, I.R., Garay, L.A., Sestric, R., Levin, D., Block, D.E., German, J.B., Boundy-Mills, K.L., 2014.
594 Oleaginous yeasts for biodiesel: current and future trends in biology and production.
595 Biotechnol. Adv. 32, 1336–1360.
596 https://doi.org/10.1016/j.biotechadv.2014.08.003
597 Sutanto, S., Zullaikah, S., Tran-Nguyen, P.L., Ismadji, S., Ju, Y.-H., 2018. *Lipomyces starkeyi*: Its current
598 status as a potential oil producer. Fuel Process. Technol. 177, 39–55.
599 https://doi.org/10.1016/j.fuproc.2018.04.012
600 Swanson, W.H., Clifton, C.E., 1948. Growth and assimilation in cultures of *Saccharomyces cerevisiae*.
601 J. Bacteriol. 56, 115–124.
602 Thorpe, R.F., Ratledge, C., 1972. Fatty acid distribution in triglycerides of yeasts grown on glucose or
603 n-alkanes. Microbiology 72, 151–163.
604 Timbo, B., Koehler, K.M., Wolyniak, C., Klontz, K.C., 2004. Sulfites—a food and drug administration
605 review of recalls and reported adverse events. J. Food Prot. 67, 1806–1811.
606 https://doi.org/10.4315/0362-028X-67.8.1806
607 Toit, W.J. du, Marais, J., Pretorius, I.S., Toit, M. du, 2006. Oxygen in Must and Wine: A review. South
608 African J. Enol. Vitic. 27, 76–94.
609 https://doi.org/10.21548/27-1-1610
610 Vally, H., Misso, N.L.A., Madan, V., 2009. Clinical effects of sulphite additives. Clin. Exp. Allergy 39,
611 1643–1651. https://doi.org/10.1111/j.1365-2222.2009.03362.x
612 Venturin, C., Boze, H., Fahrasmane, L., Moulin, G., Galzy, P., 1994. Influence de la concentration en
613 glucose et en oxygène sur la capacité fermentaire de la souche *Hanseniaspora uvarum* K5
614 (Niehaus). Sciences des Aliments 14, 321–333.
615 Vignault, A., Gombau, J., Jourdes, M., Moine, V., Canals, J.M., Fermaud, M., Roudet, J., Zamora, F.,
616 Teissedre, P.-L., 2020. Oenological tannins to prevent *Botrytis cinerea* damage in grapes and
617 musts: Kinetics and electrophoresis characterization of laccase. Food Chem. 316, 126334.
618 https://doi.org/10.1016/j.foodchem.2020.126334
619 Warner, C.R., Diachenko, G.W., Bailey, C.J., 2000. Sulfites: an important food safety issue. Food test.
620 anal. 6, 8–10.
621 Wickham, H., Chang, W., Wickham, M.H., 2016. Package 'ggplot2.' Create Elegant Data Visualisations
622 Using the Grammar of Graphics. Version 2, 1–189.
623 Wickham, H., François, R., Henry, L., Müller, K., Studio, R., 2020. dplyr: a grammar of data
624 manipulation. R Packag. version 1.0. 2.
625 Wickham, H., Wickham, M.H., 2017. Package tidyverse. Easily Install and Load the Tidyverse.
626 Windholtz, S., Dutilh, L., Lucas, M., Maupeu, J., Vallet-Courbin, A., Farris, L., Coulon, J., Masneuf-
627 Pomarède, I., 2021a. Population dynamics and yeast diversity in early winemaking stages
628 without sulfites revealed by three complementary approaches. Appl. Sci. 11, 2494.
629 https://doi.org/10.3390/app11062494
630 Windholtz, S., Nioi, C., Redon, P., Masneuf-Pomarede, I., Thibon, C., 2021b. Does bioprotection by
631 adding yeasts present antioxidant properties ? 8th edition of Macrowine virtual congress.
632 https://www.infowine.com/intranet/libretti/0/19546-
633 38%20Sara%20WINDHOLTZ%20Poster%20Macrowine%202021.pdf
634 Windholtz, S., Redon, P., Lacampagne, S., Farris, L., Lytra, G., Cameleyre, M., Barbe, J.-C., Coulon, J.,
635 Thibon, C., Masneuf-Pomarède, I., 2021c. Non-*Saccharomyces* yeasts as bioprotection in the
636 composition of red wine and in the reduction of sulfur dioxide. LWT 149, 111781.
637 https://doi.org/10.1016/j.lwt.2021.111781
638 Windholtz, S., Vinsonneau, E., Farris, L., Thibon, C., Masneuf-Pomarède, I., 2021d. Yeast and
639 Filamentous Fungi Microbial Communities in Organic Red Grape Juice: Effect of Vintage,

640 Maturity Stage, SO_2, and Bioprotection. Front. Microbiol. 12, 748416.
641 https://doi.org/10.3389/fmicb.2021.748416
642 Zott, K., Claisse, O., Lucas, P., Coulon, J., Lonvaud-Funel, A., Masneuf-Pomarede, I., 2010.
643 Characterization of the yeast ecosystem in grape must and wine using real-time PCR. Food
644 Microbiol. 27, 559–567.
645 https://doi.org/10.1016/j.fm.2010.01.006
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649 **Figure caption**

650 **Figure 1**: Oxygen Consumption Rate (OCR expressed as mg of O_2 consumed/L/h/ viable cell) of yeast
651 species
652 1 dot = 1 repetition/ strain. Kruskall-Wallis significance at 0.01, violin with different letters differ
653 significantly
654 **Figure 2**: OCR obtained for different bioprotection industrial preparations.
655 ANOVA (***: p-value<0.001), HSD post hoc, different letters indicate that means are significantly
656 different.
657 Dunnett's test, significance is indicated as follow: * significant at 0.05, ** significant at 0.01, ***
658 significant at 0.001 in comparison to the negative control (n=5) or positive control (n=5)
659 ZE: mix of *M. pulcherrima* et *T. delbrueckii* (Zymaflore®Egide), Sc: *S. cerevisiae*
660 (Zymaflore®Xpure), Mp: *Metschnikowia pulcherrima* of ZE, *Torulaspora delbrueckii*: *Torulaspora*
661 *delbrueckii* of ZE; Inoculum: mix of *Torulaspora delbrueckii* and *Metschnikowia pulcherrima* with
662 fresh biomass after pre-culture step (10^6 cell/mL), Heat inactivated: ADY ZE rehydrated then heating
663 at 80°C during 30 min.
664 **Figure 3:** Population dynamics of acetic acid bacteria during prefermentary stages.
665 BP: bioprotection ZE at 50 mg/L, Without SO_2 and SO_2 at 50 mg/L.
666 Significance is indicated as follow: * significant at 0.05, ** significant at 0.01, *** significant at 0.001
667 (ANOVA).

668 **Figure 4**: Impact of bioprotection ZE (50 mg/L) on *G. oxydans* and on O_2 consumption.
669 Population levels of *G. oxydans* at 10°C (**A**) and 15°C (**B**) UFC/mL. O_2 consumption rate (OCR: mg
670 O_2 consumed/L/h) after the first saturation (0h→26h) and the second saturation (26h→48h) at 10°C
671 and 15°C (**C**).
672 Go: *G. oxydans* at 10^5 cells/mL alone, ZEGo: *G. oxydans* at 10^5 cells/mL with 50 mg/L of ZE.
673 Significance is indicated as follow: * significant at 0.05, ** significant at 0.01, *** significant at 0.001
674 (T-test).
675 **Supplementary data caption**
676 **Figure S.1:** MSP Dendrogram by MALDI Biotyper Method Editor for *Metschnikowia pulcherrima*
677 strains
678 Default parameter set for MSP dendrogram creation (Distance Mesure: correlation), DSM 70336
679 DSM, CBS 2244 PAH, VLM and CBS 610NT CBS belong to Bruker database.
680 **Figure S.2:** Phylogenetic tree for *Metschnikowia pulcherrima* strains based on D1/D2 regions
681 comparing to reference strains by species in (Sipiczki, 2022)

682 **Figure S.3:** Process for obtaining the OCR for a strain.

683 **Table S.1**: Collection of yeast used this study.

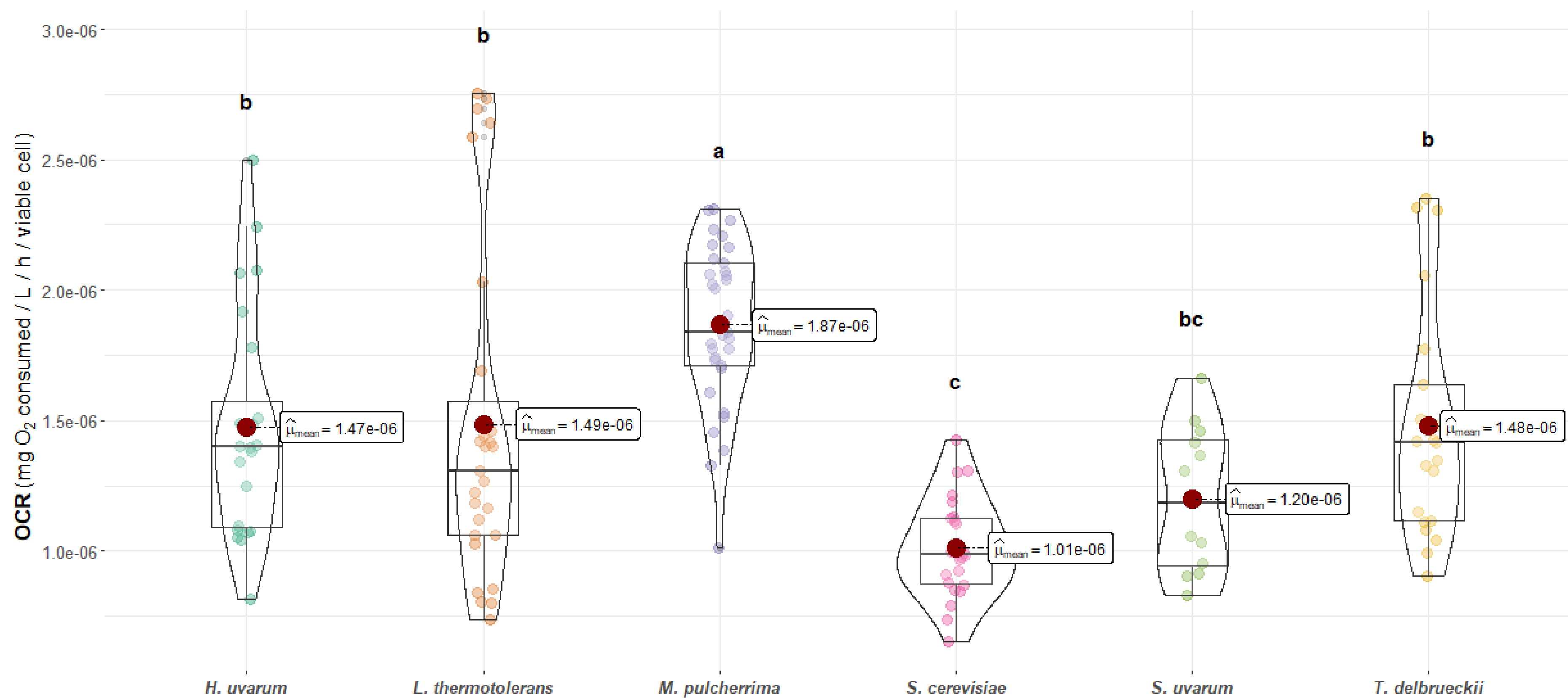
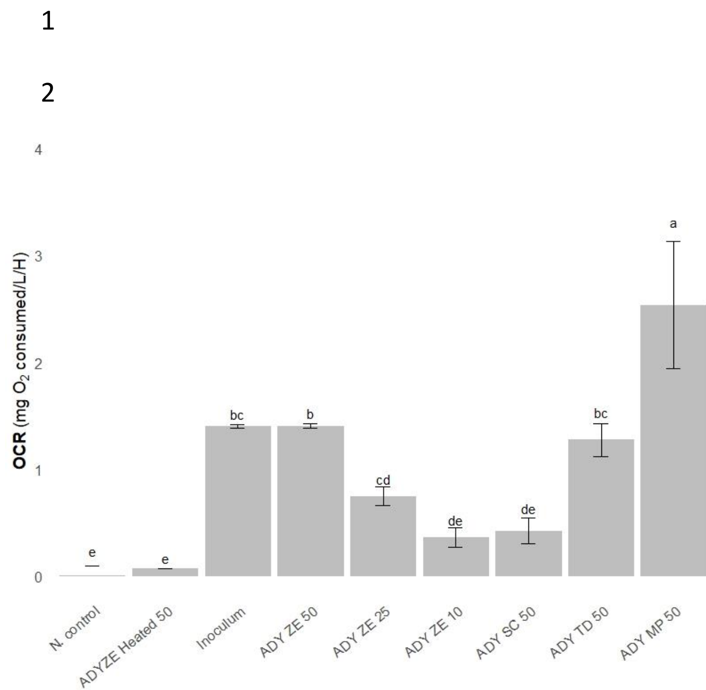


Figure 1: Oxygen Consumption Rate (OCR expressed as mg of O_2 consumed/L/h/viable cell) of yeast strains and species. (color should be used to print this figure)

1 dot = 1 repetition/ strain. Kruskall-Wallis significance at 0.01, violin with different letters differ significantly



Treatmens	Inoculation rate	Dunnett test (comparison with negative control)	Dunnett test (comparison with positive control)
Negative Control		X	***
ADYZE Heated 50	50 mg/L		***
ZE Inoculum	10^6 cells/mL	***	
ADY ZE 10	10 mg/L	*	***
ADY ZE 25	25 mg/L	***	**
Positive control ADY ZE 50	50 mg/L	***	X
ADY SC 50	50 mg/L	**	***
ADY MP 50	50 mg/L	***	***
ADY TD 50	50 mg/L	***	

Figure 2: OCR obtained for different bioprotection industrial preparations.

ANOVA (***: p-value<0.001), HSD post hoc, different letter indicate means are significantly differents. Dunnett's test, significance is indicated as follow: * significant at 0.05, ** significant at 0.01, *** significant at 0.001 in comparison with negative control (n=5) or positive control (n=5)

ZE: mix of *M. pulcherrima* et *T. delbrueckii* (Zymaflore®Egide), Sc: *S. cerevisiae* (Zymaflore®Xpure), Mp: *Metschnikowia pulcherrima* of ZE, *Torulaspora delbrueckii*: *Torulaspora delbrueckii* of ZE; Inoculum: mix of *Torulaspora delbrueckii* and *Metschnikowia pulcherrima* with fresh biomass after pre-culture step (10^6 cell/mL), Heat inactivated: ADY ZE rehydrated then heating at 80°C during 30 min.

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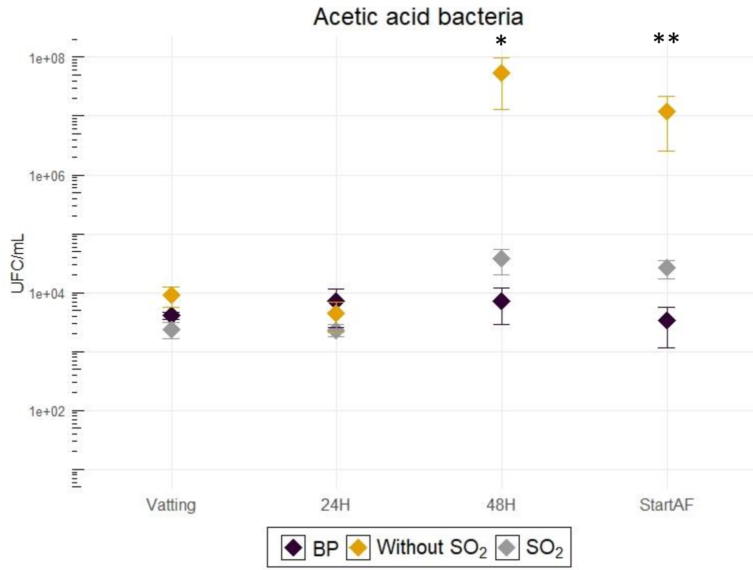

41 **Figure 3:** Population dynamics of acetic acid bacteria during prefermentary stages. (color should be used to
42 print this figure)
43 BP: bioprotection ZE at 50 mg/L, Without SO_2 and SO_2 at 50 mg/L .
44 Significance is indicated as follow: * significant at 0.05, ** significant at 0.01, *** significant at 0.001
45 (ANOVA).

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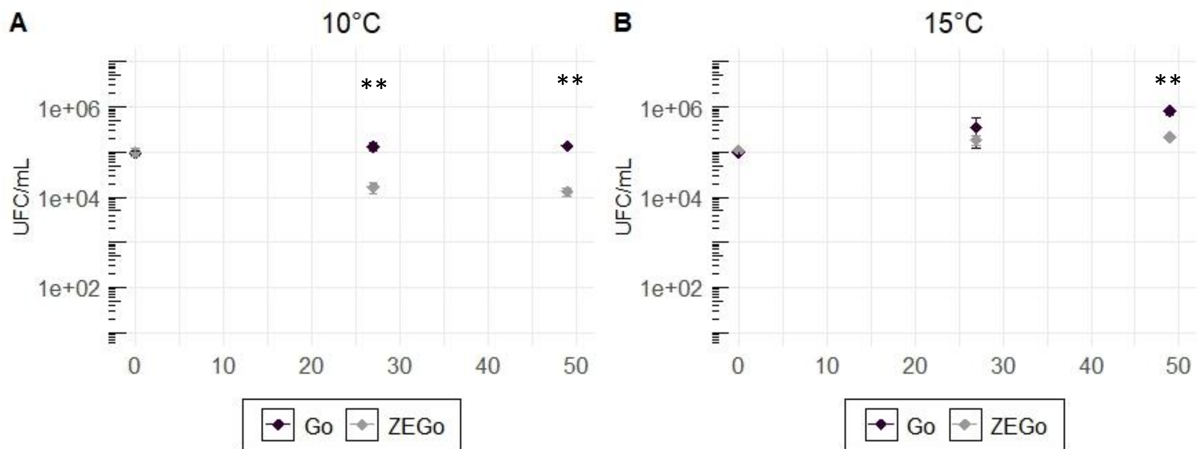

	OCRs at **10°C** (mg O_2 consumed/L/H)		OCRs at **15°C** (mg O_2 consumed/L/H)	
Treatment	OCR (0h→26h)	OCR (26h →48h)	OCR (0h→26h)	OCR (26h →48h)
Go	0.010 ± 0.023 ***	0,060±0.0035**	0.18 ±0.005**	0.22±0.136*
ZEGo	0.822 ±0.018	2.432±0.068	2.17 ±0.136	4.72±0.003

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56 **Figure 4**: Impact of bioprotection ZE (50 mg/L) on *G. oxydans* and on O_2 consumption

57 Population levels of *G. oxydans* at 10°C (**A**) and 15°C (**B**) UFC/mL. O_2 consumption rate (OCR: mg O_2
58 consumed/L/H) after the first saturation (0h→26h) and the second saturation (26h→48h) at 10°C and 15°C
59 (**C**).

60 Go: *G. oxydans* at 10^5 cells/mL alone, ZEGo: *G. oxydans* at 10^5 cells/mL with 50 mg/L of ZE.
61 Significance is indicated as follow: * significant at 0.05, ** significant at 0.01, *** significant at 0.001 (T-
62 test).

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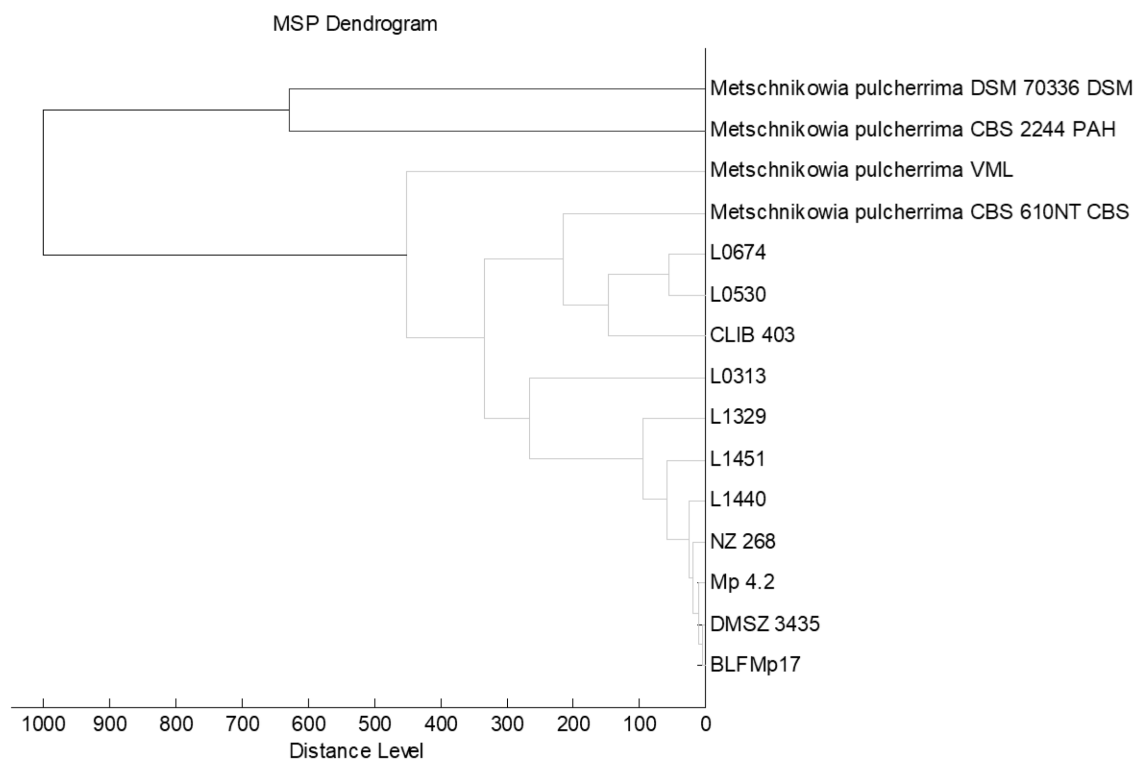
72 **Supplementary data**



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74 **Figure S.1: MSP Dendrogram by MALDI Biotyper Method Editor for *Metschnikowia pulcherrima***
75 **strains**

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77 DSM, CBS 2244 PAH,VLM and CBS 610NT CBS belong to Bruker database.

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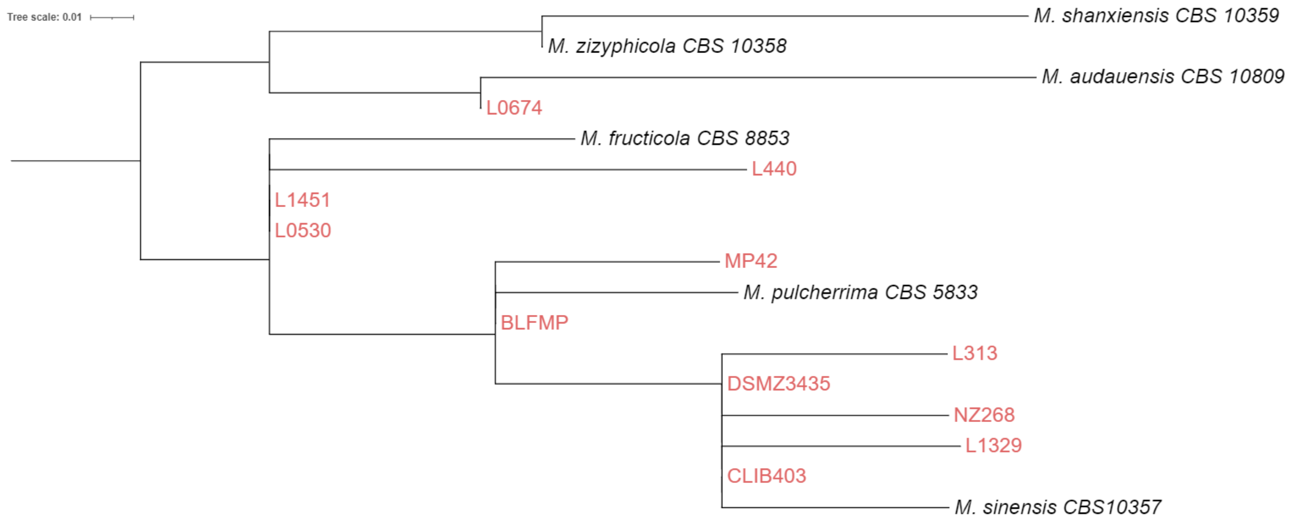

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81 **Figure S.2: Phylogenetic tree for *Metschnikowia pulcherrima* strains based on D1/D2 regions comparing**
82 **to reference strains by species in** (Sipiczki, 2022)

83 In black, reference strains for *Metschnikowia species (Sipiczki, 2022), in pink color strain of own collection.*
84 *Sequences treatment was obtained using BioEdit Sequence alignement editor logiciel and*
85 http://phylogeny.lirmm.fr/ *to generate tree.*

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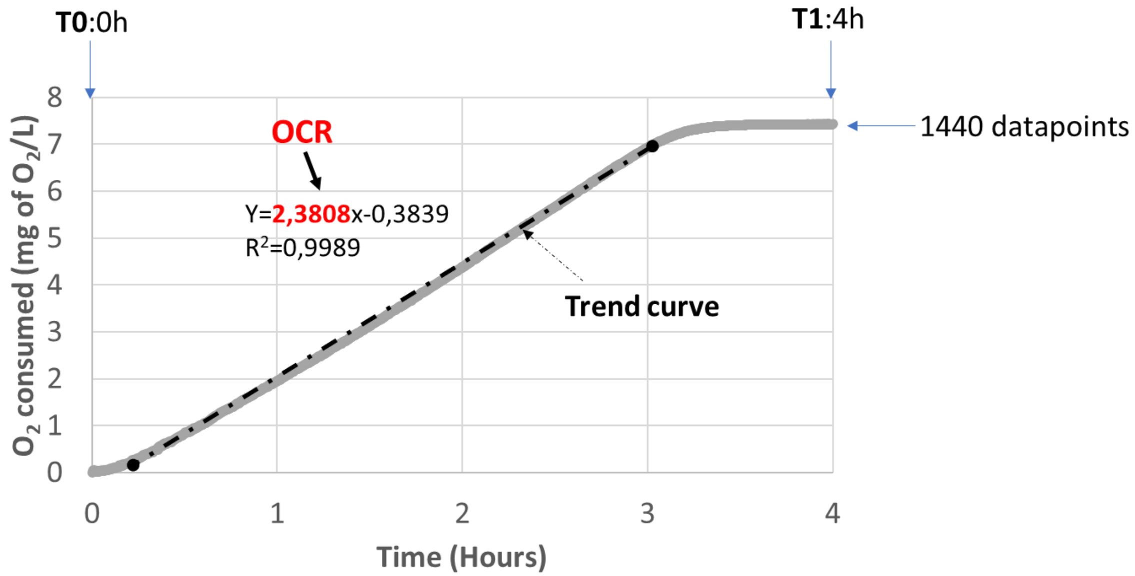

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92 **Figure S.3:** Process for obtaining the OCR for a strain.

93 **T0**: start of the experiment (0h) ; **T1**: end of the experiment, than all O_2 dissolved was consumed; curve
94 acquisition (in grey) was obtained by a compact 4 channel FireStingO2 oximeter (Pyroscience, Bionef, France)
95 with optics fiber and sensor spot OXSP5 (Bionef, Montreuil, France) (in this example, 1440 points make it up);
96 trend curve was obtained by Microsoft Excel (2015). The coefficient of the curve trend is OCR of experiment
97 (in red on the graph).

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Table S.1: Yeast collection of used this study

CRBO - Centre de Ressources Biologiques OEnologie, France; **DBVPG:** The Industrial Yeasts Collection DBVPG, Italy; **ISVV** – Institut des Sciences de la Vigne et du Vin, France; **MUCL** – Mycothèque de l'Université catholique de Louvain, Belgium; **NRRL**/ARS – NRRL Agriculture Research Service Culture collection, USA; **CRPR** – Centre de Recherche Pernod-Ricard, France; **UNIFG** – University of Foggia; **UWOPS** – Culture Collection of the University of Western Ontario; **DSMZ** – Leibniz-Institut DSMZ, Braunschweig, Germany; **CLIB** – Collection de Levures d'Intérêt Biotechnologique, CIRM-Levures, INRA/AgroParisTech, Thiverval-Grignon, France ; **UOA**/HCPF: Hellenic Collection of Pathogenic Fungi, University of Athens ; **UWOPS** - Culture collection of the University of Western Ontario ; **CBS** : Fungal Biodiversity Centre, Centraalbureau voor Schimmelcultures, institute of the Royal Netherlands Academy of Arts and Sciences (Koninklijke Nederlandse Akademie van Wetenschappen)

NA stands for "Not Available."

1. Albertin W, Setati ME, Miot-Sertier C, Mostert TT, Colonna-Ceccaldi B, Coulon J, Girard P, Moine V, Pillet M, Salin F, Bely M, Divol B, Masneuf-Pomarede I. 2016. Hanseniaspora uvarum from Winemaking Environments Show Spatial and Temporal Genetic Clustering. Front Microbiol 6.
2. Hranilovic A, Bely M, Masneuf-Pomarede I, Jiranek V, Albertin W. 2017. The evolution of Lachancea thermotolerans is driven by geographical determination, anthropisation and flux between different ecosystems. PLoS One 12:e0184652.
3. Freel KC, Charron G, Leducq J-B, Landry CR, Schacherer J. 2015. Lachancea quebecensis sp. nov., a yeast species consistently isolated from tree bark in the Canadian province of Québec. Int. J. Syst. Evol. Microbiol. 65:3392–3399.
4. Albertin W, Chasseriaud L, Comte G, Panfili A, Delcamp A, Salin F, Marullo P, Bely M. 2014. Winemaking and bioprocesses strongly shaped the genetic diversity of the ubiquitous yeast Torulaspora delbrueckii. PLoS One 9:e94246.
5. Albertin W, Chernova M, Durrens P, Guichoux E, Sherman DJ, Masneuf-Pomarede I, Marullo P. 2018. Many interspecific chromosomal introgressions are highly prevalent in Holarctic Saccharomyces uvarum strains found in human-related fermentations. Yeast 35:141–156.

Strains	Species	Genetic groups	Geographical origins	collection	Ref.
NZ15	*H. uvarum*	Group A	New Zealand (Grape/wine)	CRPR	(1)
L1433		Group B	France (Grape/wine)	CRBO	(1)
y-1614		Group C	Russia (Grape/wine)	NRRL	(1)
DSMZ70285		NA	Germany (Nature, soil)	DSMZ	(1)
L0551		Group A-B-C	France (Grape/wine)	CRBO	(1)
L1448		Group B	Gironde, France (Grape/wine)	CRBO	(1)
y-915		NA	NA (Cider)	NRRL	(1)
yb-3199		NA	Mason, Michigan, USA (Rotten apples)	NRRL	(1)

CONCERTO™	*L. thermotolerans*	Domestic 1	« Mediterranean country »	CHR Hansen	(2)
UNIFG 18		Domestic 2	Italy *(*Grape/wine)	UNIFG	(2)
L0672		Mix Europe/N. America	Gironde, France (Grape/wine)	CRBO	(2)
NRLL Y-27911		NA	Louisiana, USA (insect)	NRRL	(3)
ZYMAFLORE ® OMEGA (Lt7)		NA	(Grape/wine)	LAFFORT	
UWOPS 91-902.1		Hawaii/California	Hawaii, Saddle Rd Park (plant)	A.LS	(3)
DBVPG 6867		NA	Brazil (plant)	DBVPG	(3)
Clib 292		Domestic 1	Russia (fruit)	CLIB	(2)
Ltyq25		Europe/oak/France grapes	Gironde, France (Grape/wine)	ISVV	(2)
L1440	*M. pulcherrima*	NA	Gironde, France (Grape/wine)	CRBO	
L1329		NA	Gironde, France (Grape/wine)	CRBO	
L0674		NA	Gironde, France (Grape/wine)	CRBO	
DSMZ3435		NA	USA-LA (*zobellii* Seawater)	CRBO	
Clib403		NA	Egypte - (Fruit)	CRBO	
L0313		NA	Gironde, France (Grape/wine)	CRBO	
L 0530		NA	Gironde, France (Grape/wine)	CRBO	
BLFMP17		NA	France (Grape/wine)	LAFFORT	
Mp4.2		NA	France (Grape/wine)	LAFFORT	
L1451		NA	Gironde, France (Grape/wine)	CRBO	
NZ 268		NA	New Zealand (Grape/wine)	CRPR	
ZYMAFLORE® X5	*S. cerevisiae*	NA	(Grape/wine)	LAFFORT	
ZYMAFLORE® Xpure		NA	(Grape/wine)	LAFFORT	
Zymaflore® Fx10		NA	Gironde, France (Grape/wine)	LAFFORT	
ZYMAFLORE® F15		NA	Gironde, France (Grape/wine)	LAFFORT	
ACTIFLORE® F33		NA	Gironde, France (Grape/wine)	LAFFORT	
ACTUFLORE® 522 Davis		NA	(Grape/wine)	LAFFORT	
EXCELLENCE® XR		NA	(Grape/wine)	LAMOTHE ABIET	
ZYMAFLORE® VL2		NA	(Grape/wine)	LAFFORT	
B172	*T. delbrueckii*	Grape/Wine	Crete, Greece (Grape/Wine)	UOA	(4)
Clib230		Nature America	NA	CLIB	(4)
L0705		Bioprocess	Gironde, France (Grape/wine)	CRBO	(4)
MUCL51641		Nature Old World	France (Paste of concentrated fruit)	MUCL	(4)
y-11747		Dairy	USA, Ohio, Columbus (Spoilaged sweetened condensed milk)	NRRL	(4)
L1548		Grape/Wine	Cauquenes, Chile (Grape/Wine)	CRBO	
Zymaflore® alpha		NA	(Grape/wine)	LAFFORT	
CBS425	*S. uvarum*	NA	Switzerland (Cider)	CBS	(5)
CBS377		NA	Germany (Cider)	CBS	(5)
BR6-2		NA	Brittany/Normandy, France (Cider)	NA	(5)
D11		NA	France (Sparkling wine)	NA	(5)

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135 **Tableau S.2:** : Analysis of wine after alcoholic fermentation. Bioprotection (BP), SO_2 (50 mg.L-1) and
136 Without SO_2 treatments in early stages of winemaking.

Modality	Ethanol (v/v)	Residual sugars (g/L)	Total acidity (tartaric acid g/L)	Malique acid (g/L)	PH	Volatile acidity (acetic acid g/L)
Bioprotection	12. 45 ± 0.16	2.7 ± 0.28	6,4 ± 0	1,3 ± 0.07	3,66 ± 0	0,25 ± 0
Without SO_2	12,53 ± 0.02	2 ± 0	6,4 ± 0	1.05 ± 0.35	3,68 ± 0	0,25 ± 0.01
SO_2	12.57 ± 0.06	3.65 ± 1.3	6.35 ±0.32	1.4 ± 0	3.59 ± 0	0,25 ± 0

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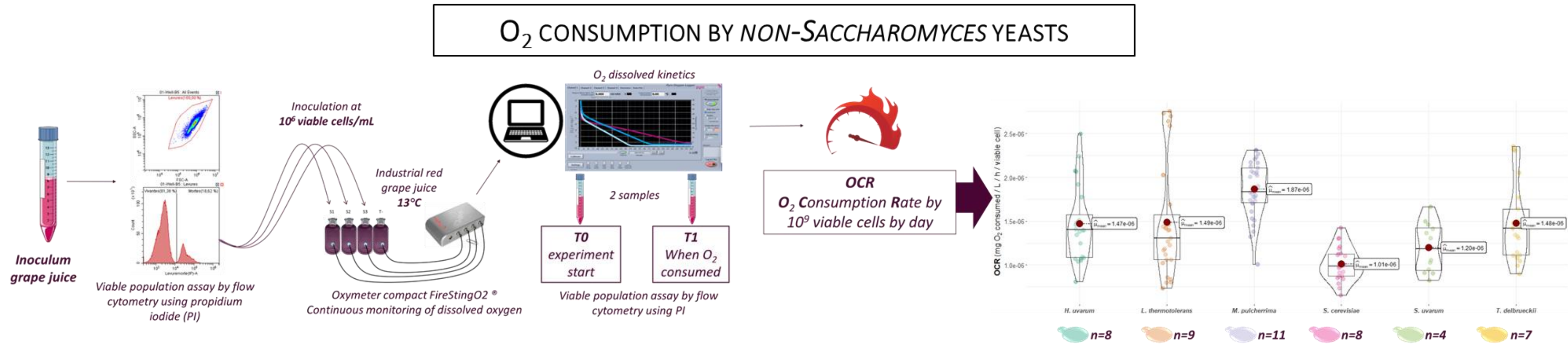
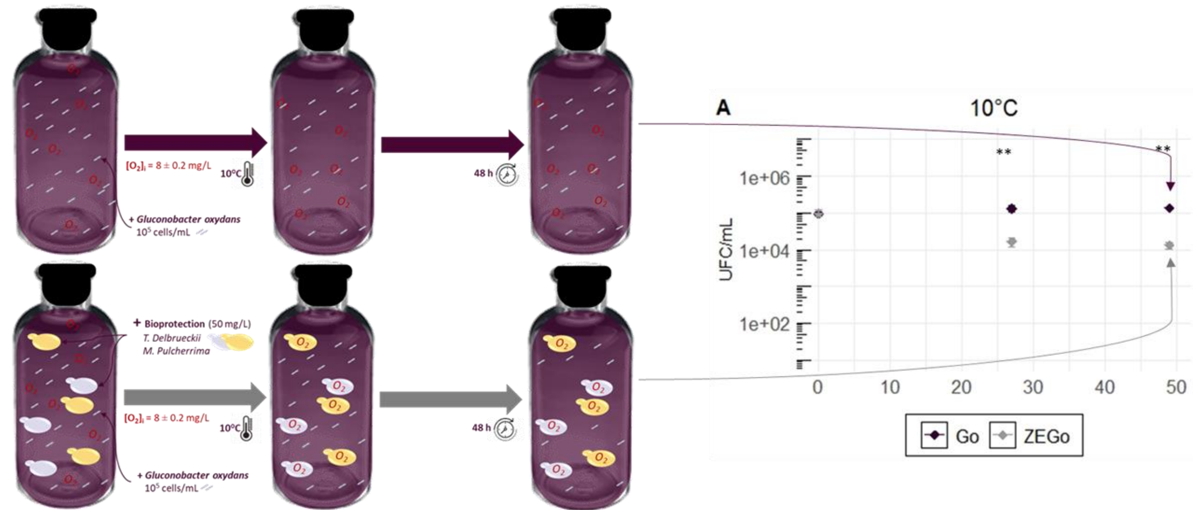
140 GRAPHICAL ABSTRACT (COLOR SHOULD BE USED TO PRINT THIS FIGURE)



IMPACT OF NON-SACCHAROMYCES YEASTS ON ACETIC ACID BACTERIA



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