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Review

Applications of *Metschnikowia pulcherrima* in Wine Biotechnology

Antonio Morata, Iris Loira *, Carlos Escott, Juan Manuel del Fresno, María Antonia Bañuelos and José Antonio Suárez-Lepe

Departamento de Química y Tecnología de Alimentos, Universidad Politécnica de Madrid, Av. Puerta de Hierro, nº 2, 28040 Madrid, Spain

* Correspondence: iris.loira@upm.es

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Abstract: *Metschnikowia pulcherrima* (*Mp*) is a ubiquitous yeast that frequently appears in spontaneous fermentations. The current interest in *Mp* is supported by the expression of many extracellular activities, some of which enhance the release of varietal aromatic compounds. The low fermentative power of *Mp* makes necessary the sequential or mixed use with *Saccharomyces cerevisiae* (*Sc*) to completely ferment grape musts. *Mp* has a respiratory metabolism that can help to lower ethanol content when used under aerobic conditions. Also, *Mp* shows good compatibility with *Sc* in producing a low-to-moderate global volatile acidity and, with suitable strains, a reduced level of H_2S. The excretion of pulcherrimin gives *Mp* some competitive advantages over other non-*Saccharomyces* yeasts as well as providing some antifungal properties.

Keywords: *Metschnikowia pulcherrima*; oenological uses; enzymes; stable pigments; pulcherrimin

1. Ecology and Physiology

Metschnikowia pulcherrima (*Mp*) is a globous/elliptical yeast that cannot be distinguished from *Saccharomyces cerevisiae* (Sc) by microscopy (Figure 1). Sometimes, it can be observed a single large, highly refractive oil droplet inside the cell. *Mp* is a teleomorph yeast belonging to an ascomycetous genus [1]. Its anamorph form is called *Candida pulcherrima*. *Mp* is a ubiquitous yeast that has been found in grapes, fruits (fresh and spoiled), flowers, nectars and tree sap fluxes. Several insects can work as vectors for this yeast. *Mp* strains can be identified through the use of selective and differential substrates; *Mp* strains showed both positive β-glucosidase enzyme activity and proteolytic activity [2]. *Mp* grows properly in either YPD or L-lysine media, and it can also can use arbutin as a carbon source in agar plates, indicating the expression of β-glucosidase activity (Figure 2) [3]. Recently, its nitrogen requirement was evaluated and slower consumption rates of ammonium were observed in *Mp* in comparison to other yeast genera [4]. This slow nitrogen uptake is indicative of its low fermentative ability [5].

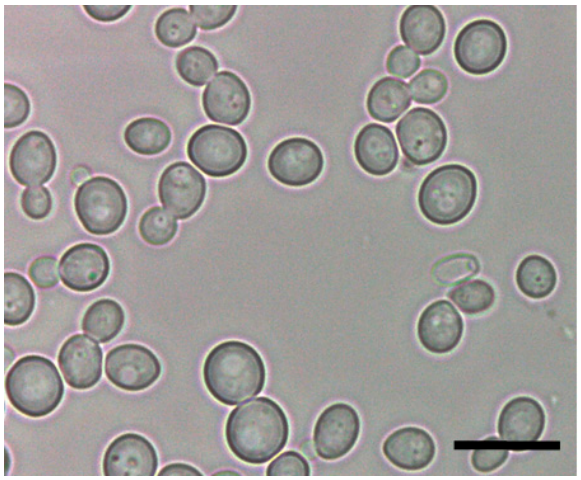

Figure 1. Cell morphology and shape of *Metschnikowia pulcherrima*. Graphical scale 10 μm.

The β-glucosidase activity related to *Mp* has been associated with different intracellular β-glucosidases, with the identification of three different bands observed when using fluorogenic substrates via an electrophoretic technique [6]. Of these three bands, the major band has similar physicochemical properties to those found in other studied yeasts, with high activity in ethanol and glucose concentrations often found in wines but low stability below pH 4. *Mp* is unable to develop in YPD at 37 °C and shows very weak or no growth in nitrate agar (Figure 2). It is able to use glucose, sucrose, fructose, galactose and maltose as carbon sources but shows weak or inexistent development in lactose [7]. It can grow properly under low temperature (15–20 °C) and pH conditions (3–6) [8]. Under environmental stress conditions such as a shortage of nitrogen, its recognition in optical microscopy is easy thanks to the appearance of a fat globule inside the cell at the beginning of the sporulation process [8]. In its sporulated form, the asci of *Metschnikowia* are long and clavate, containing one to two acicular to filiform spores [1].

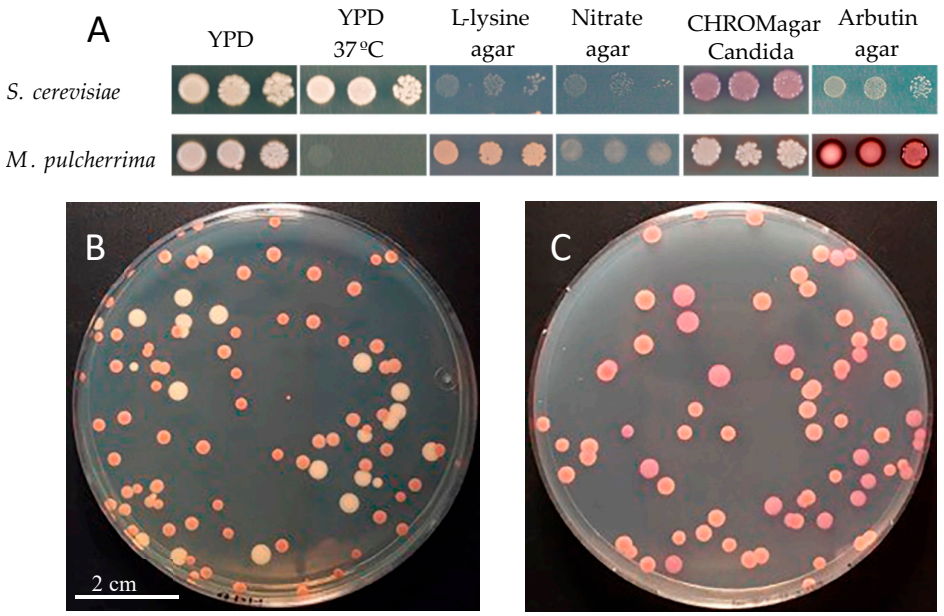

Figure 2. (**A**) Development and colony appearance in several growth media and different culture conditions (temperature). (**B**) *Metschnikowia pulcherrima* (*Mp*) orange colonies, some of them surrounded with white halos and *Saccharomyces cerevisiae* (*Sc*) white/creamy colonies in YPD media. (**C**) *Mp* and *Sc* in CHROMagar® media. Sc: bigger colonies with light pink color, *Mp*: smaller orange colonies, some of them with white halos.

The fermentative power of *Mp* is low, with many strains easily reaching 4% *v/v* in ethanol [3], although previous studies have observed the production of ethanol up to 6–7% *v/v* [9]. This feature, together with the fact that the presence of *Mp* in freshly pressed must is about 19–39% of the yeast ecology [9], makes it necessary to use *Mp* together with other yeast with a high fermentative power such as *Sc* or *Schizosaccharomyces pombe* to fully ferment grape sugars [10]. Its volatile acidity is also quite moderate, ranging from 0.3 to 0.4 g/L expressed as acetic acid [3]. Moreover, some strains are able to decrease the formation of H_2S during fermentation [11].

The fermentative performance of *Mp* is lower than that observed for other non-*Saccharomyces* species. The CO_2 production during fermentation yielded lower amounts for *Mp* than for *Sc* with 4.5 g per 100 mL vs. 12.9 g per 100 mL, respectively [12]. *Mp* has an intermediate acetoin production during alcoholic fermentation with respect to other species, such as *S. cerevisiae* and *B. bruxellensis* with low acetoin production and *C. stellata* and *K. apiculata* with the highest production of acetoin. The metabolic pathway for the production of this secondary metabolite from fermentation is shown in Figure 3. In addition, the amount of 2,3-butanediol produced by *Mp* is usually lower than that produced by *Sc*.

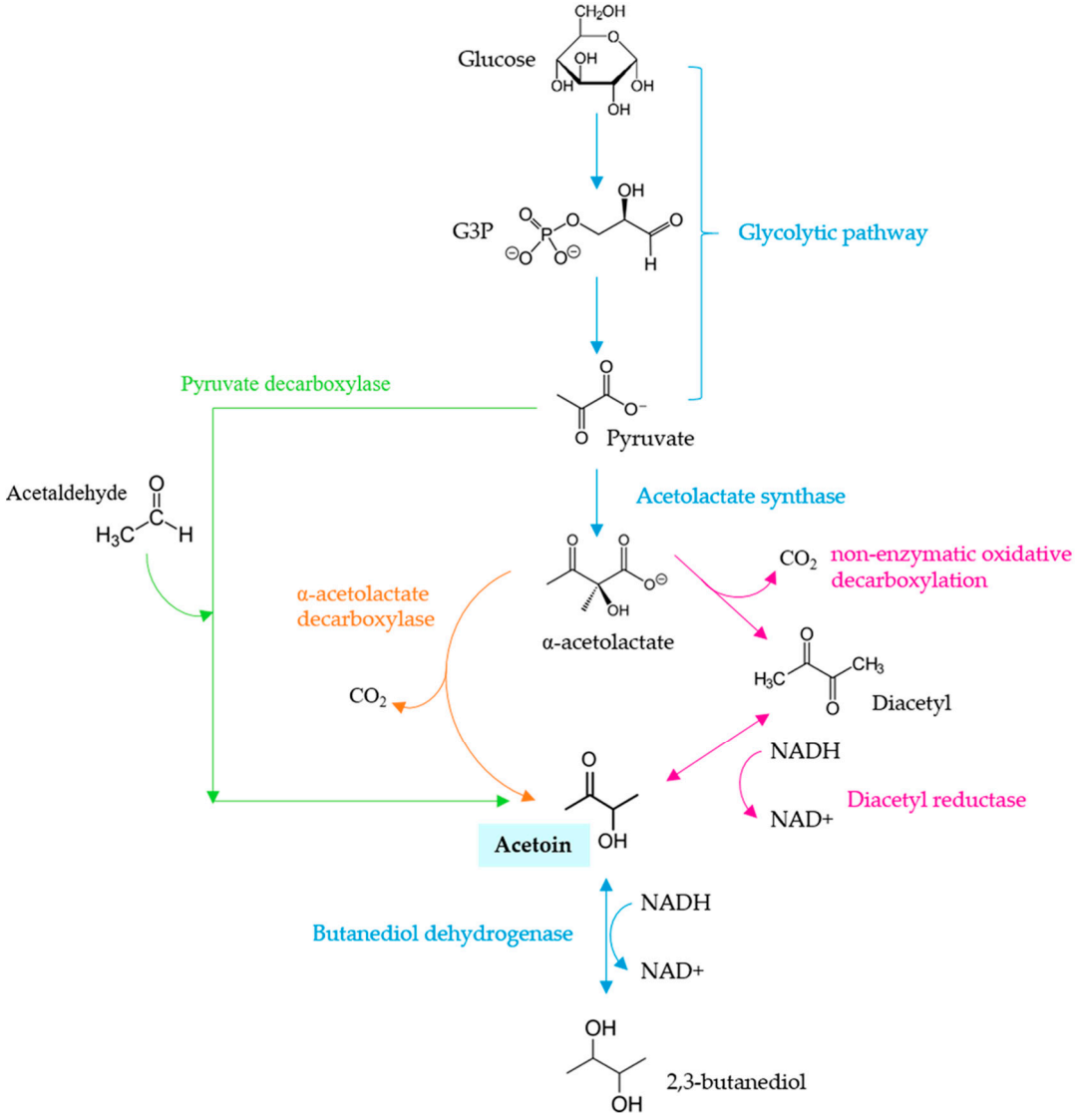

Figure 3. Metabolic route for the biosynthesis of acetoin by yeasts (adapted from Romano and Suzzi, [13]).

In mixed cultures with *S. cerevisiae*, viability was found to decrease rapidly after a few days of fermentation because of the low resistance to the ethanol produced by *S. cerevisiae* [14,15]. The use of emerging physical technologies that are able to strongly reduce the wild yeast content in grapes [16] can facilitate the prevalence of *Mp* during a longer period until the sequential inoculation of Sc, thus also increasing its effect on the sensory profile of the wines.

The sensibility of *Mp* to SO_2 is lower than that observed in Sc, *Saccharomycodes ludwigii* or *S. pombe*, but *Mp* shows a medium resistance compared with other non-*Saccharomyces* species [7]. A certain sensibility to some antimicrobials such as carvacrol and thymol has also been observed [17]. Regarding the use of dimethyl dicarbonate (DMDC), the growth of *Mp* strains during the fermentation of grape must is delayed, but not inhibited, after the addition of 400 mg/L DMDC [18]. The total inhibition of the microbial population can be achieved with 500 mg/L of DMDC. *Sc* can survive the addition of 200 mg/L DMDC, whereas the growth of other species of the genus *Saccharomyces* is inhibited with 150 mg/L DMDC.

2. Antimicrobial Bio-Tool

Mp can be used as a biological control agent thanks to its ability to produce natural antimicrobial compounds, namely pulcherrimin, an insoluble red pigment with antifungal activity. This peculiar antimicrobial activity is produced by the depletion of iron in the medium through the precipitation of iron(III) ions caused by the interaction with pulcherriminic acid, a precursor of pulcherrimin secreted by *Mp*. In this way, the environment becomes inhospitable to other microorganisms that require iron for their development. Pulcherrimin has shown effective inhibitory activity against several yeasts: *Candida tropicalis* and *Candida albicans*, as well as the *Brettanomyces/Dekkera*, *Hanseniaspora* and *Pichia* genera; and fungi: *Botrytis cinerea*, as well as *Penicillium*, *Alternaria* and *Monilia* spp. [19–24]. However, *S. cerevisiae* seems not to be affected by this antimicrobial activity [21,22]. Therefore, the use of *Mp* as a selected starter in sequential or mixed biotechnologies with *Sc* could be of great interest in modern enology.

Mp, as well as other yeast species such as *Wickerhamomyces anomala* (formerly *Pichia anomala*) and *Torulaspora delbrueckii* (*Td*), has a broad killer spectrum against some spoilage yeasts [25,26], of which *C. glabrata* had the highest sensitivity against the toxins from this species [27]. *Mp* has also been described as biofungicide capable of effectively reducing the incidence of *Botrytis* development in postharvest fruits [28]. Its antagonistic mechanism is mainly based on its competition for nutrients [29].

3. Aroma Compounds

The single use of *Mp* has led to excessive production of ethyl acetate with negative sensory repercussions [30]. However, the mixed use of *Mp* with *Saccharomyces uvarum* reduces the production of ethyl acetate, simultaneously favoring the formation of 2-phenyl ethanol and 2-phenylethyl acetate [30]. The use of co-inoculations of this type (mixed fermentations with *Mp*/*Sc*) has produced high contents of acetate esters and β-damascenone with lower levels of C_6 alcohols in ice wines made from the Vidal blanc grape variety [31]. An improvement in the aromatic complexity of the wines can be obtained by the use of *Mp* as a co-starter with *Sc* [3,32], mainly due to its high production of esters derived from its intense extracellular enzymatic activity [10,33]. Similarly, sequential fermentations with *Mp* showed a higher production of higher alcohols, with particularly high concentrations of isobutanol and phenylethanol [4].

4. Enzymatic Activities

Activities of the following enzymes have been described in *Mp*: pectinase, protease, glucanase, lichenase, β-glucosidase, cellulase, xylanase, amylase, sulphite reductase, lipase and β-lyase [11,33–35]. This is because *Mp* one of the non-*Saccharomyces* yeast species able to express more extracellular hydrolytic enzymes. Its high proteolytic activity makes it a very interesting fermentation partner for *Sc*, since the amino acids released (including those from autolysis) can serve as a source of nutrients for

Sc [36]. In addition, its intense glucosidase activity [2], higher under aerobic conditions [37], promotes the release of varietal aromas from the grape by hydrolyzing bound monoterpenes. However, it is important to always remember that the intensity of the enzymatic activity depends not only on the species, but also on the strain [32].

Concerning aroma enhancement, the expression of β-D-glucosidase favors the release of free terpenes and this activity has been evaluated using the substrates 4-methylumbelliferyl-β-D-glucoside (MUG) and *p*-nitrophenyl-β-D-glucoside (*p*NPG), showing a good intensity with medium-to-low degradation of color by the effect on anthocyanin glucosides [38]. The commercial *Mp* L1781 (Flavia™ MP346, Lallemand) expresses α-arabinofuranosidase; this activity helps to release precursors of volatile terpenes [39,40] (Figure 4) and thiols [32,41], which help to enhance fruity smells in some varieties. This strain has shown an enzymatic specific activity of 0.22 U/mg when used as a dry yeast or fresh culture [41]. This has been measured by the hydrolysis of 11 µmol de *p*-nitrophenyl-α-L-arabinofuranosidase (*p*NPA) per minute [42].

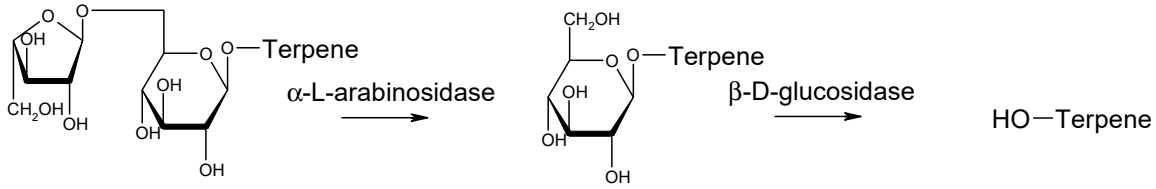

Figure 4. Effect of sequential α-arabinofuranosidase and β-D-glucosidase activities on the transformation of bonded terpenes into free forms, enhancing the aromatic profile.

Intracellular β-glucosidase of *Mp* has been purified by ion-exchange chromatography on amino agarose gel [6] and subsequently characterized. The optimum catalytic activity was observed at 50 °C and pH 4.5. The enzyme shows hydrolytic activity on β-(1→4) and β-(1→2) glycosidic bonds. The stability in alcoholic media (12% *v*/*v*) is good but it is affected by low pH.

5. Aerobic Metabolism/Alcohol Degree Reduction

The sequential use of *Mp* and *Sc* has proved to be somewhat effective in lowering the ethanol content of wine [11,43–46]. This is connected with the aerobic respiratory metabolisms of *Mp* that, in suitable aeration conditions, can aerobically metabolize more than 40% of sugars, thus significantly reducing the ethanol yield. An example of this application can be seen in the study developed by Contreras et al. (2014), where an average reduction in the alcoholic strength of 1.6% *v*/*v* was achieved when *Mp* was used in sequential fermentation with *Sc* (inoculated on the fourth day) in the production of red wine of the Syrah variety from a must with 240 g/L of sugars (potential alcoholic strength of 14% *v*/*v*). Therefore, the use of certain non-*Saccharomyces* yeast species, such as *Mp*, has been suggested as a biotechnological strategy aimed at producing wines with lower levels of ethanol [47]. In this last study, a kind of "collaboration" was seen between populations of *Mp* and *S. uvarum*, that is, a synergistic effect, achieving a lower ethanol production than in pure fermentations with each yeast. Recently, Mestre Furlani et al. (2017) evaluated the metabolic behavior of different non-*Saccharomyces* native yeasts to reduce the ethanol content during winemaking. They report that two out of the three strains of *Mp* isolated from grapes have a sugar to ethanol conversion ratio greater than >19 g/L/% *v*/*v* [48]. This confirms the usefulness of *Mp* to obtain wines with lower ethanol content.

6. Improvement of Wine Color Stability

Some non-*Saccharomyces* adsorb lower contents of anthocyanins during fermentation than *Sc* [49]. In *Sc*, the adsorption can range between 1 and 6% in total content of anthocyanins [50], but can reach up to 30% for some specific anthocyanins [51]. Adsorption is influenced by the composition and structure of the yeast cell wall. *Mp* shows a low adsorption of anthocyanins in cell walls when compared with

other yeasts such as *Sc*, *Td* or *Lachancea thermotolerans* (*Lt*) in grape skin agar (Figure 5), according to the methodology described by Caridi et al. [52].

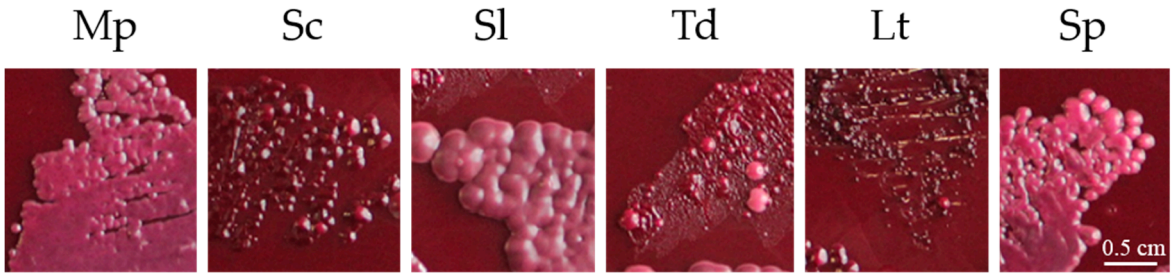

Figure 5. Adsorption of grape anthocyanins in yeast cell walls (*Saccharomyces* and non-*Saccharomyces*) during growth in a specific plating medium containing pigments. *Metschnikowia pulcherrima* (*Mp*), *Saccharomyces cerevisiae* (*Sc*), *Saccharomycodes ludwigii* (*Sl*), *Torulaspora delbrueckii* (*Td*), *Lachancea thermotolerans* (*Lt*), *Schizosaccharomyces pombe* (*Sp*).

The effect of *Mp* in the formation of stable pigments (pyranoanthocyanins and polymers) during fermentation has been studied in sequential fermentations with *Sc* and *S. pombe* [10].

7. Conclusions

The versatility of *Metschnikowia pulcherrima* lies in its ability to ferment in combination with other yeast species as well as modulate the synthesis of secondary metabolites of fermentation to improve the sensory profile of the wine. It is characterized by a medium fermentation power and a high enzymatic capacity to release aromatic precursors from the grape. In addition, this yeast has potential as a biocontrol agent in order to limit competition with other yeasts in the fermentation medium.

The abovementioned applications and features of *Metschnikowia pulcherrima* may be of great interest in order to address one of the major concerns in today's winemaking industry, such as excessive alcoholic strengths and the increasing prevalence in the market of flat wines from a sensory point of view. *Mp* could help solve these issues. The only important thing is to select the proper combination, as well as the right time and ratio of inoculation, between *Mp* and another yeast species capable of completing the alcoholic fermentation.

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References

1. Kurtzman, C.P.; Fell, J.W. *The Yeasts: A Taxonomic Study*, 4th ed.; Elsevier Science Publishers: Amsterdam, The Netherlands, 1998; ISBN 9780444813121.
2. Fernández, M.; Ubeda, J.F.; Briones, A.I. Typing of non-*Saccharomyces* yeasts with enzymatic activities of interest in wine-making. *Int. J. Food Microbiol.* **2000**, *59*, 29–36. [CrossRef]
3. Comitini, F.; Gobbi, M.; Domizio, P.; Romani, C.; Lencioni, L.; Mannazzu, I.; Ciani, M. Selected non-*Saccharomyces* wine yeasts in controlled multistarter fermentations with *Saccharomyces cerevisiae*. *Food Microbiol.* **2011**, *28*, 873–882. [CrossRef] [PubMed]
4. Prior, K.J.; Bauer, F.F.; Divol, B. The utilisation of nitrogenous compounds by commercial non-*Saccharomyces* yeasts associated with wine. *Food Microbiol.* **2019**, *79*, 75–84. [CrossRef] [PubMed]
5. Ribéreau-Gayon, P. *Handbook of Enology, Volume 1: The Microbiology of Wine and Vinifications*; John Wiley & Sons: Hoboken, NJ, USA, 2006; Volume 1, ISBN 9780470010341.

6. González-Pombo, P.; Pérez, G.; Carrau, F.; Guisán, J.M.; Batista-Viera, F.; Brena, B.M. One-step purification and characterization of an intracellular β-glucosidase from *Metschnikowia pulcherrima*. *Biotechnol. Lett.* **2008**, *30*, 1469–1475. [CrossRef] [PubMed]
7. Loira, I.; Morata, A.; Bañuelos, M.A.; Suárez-Lepe, J.A. Isolation, selection and identification techniques for non-*Saccharomyces* yeasts of oenological interest. In *Biotechnological Progress and Beverage Consumption*; Volume 19: The Science of Beverages; Grumezescu, A., Holban, A.-M., Eds.; Elsevier Academic Press: Cambridge, MA, USA, Chapter 15, in press.
8. Santamauro, F.; Whiffin, F.M.; Scott, R.J.; Chuck, C.J. Low-cost lipid production by an oleaginous yeast cultured in non-sterile conditions using model waste resources. *Biotechnol. Biofuels* **2014**, *7*, 34. [CrossRef] [PubMed]
9. Combina, M.; Elía, A.; Mercado, L.; Catania, C.; Ganga, A.; Martinez, C. Dynamics of indigenous yeast populations during spontaneous fermentation of wines from Mendoza, Argentina. *Int. J. Food Microbiol.* **2005**, *99*, 237–243. [CrossRef] [PubMed]
10. Escott, C.; Del Fresno, J.M.; Loira, I.; Morata, A.; Tesfaye, W.; del Carmen González, M.; Suárez-Lepe, J.A. Formation of polymeric pigments in red wines through sequential fermentation of flavanol-enriched musts with non-*Saccharomyces* yeasts. *Food Chem.* **2018**, *239*, 975–983. [CrossRef]
11. Barbosa, C.; Lage, P.; Esteves, M.; Chambel, L.; Mendes-Faia, A.; Mendes-Ferreira, A.; Barbosa, C.; Lage, P.; Esteves, M.; Chambel, L.; et al. Molecular and phenotypic characterization of *Metschnikowia pulcherrima* strains from Douro wine region. *Fermentation* **2018**, *4*, 8. [CrossRef]
12. Romano, P.; Granchi, L.; Caruso, M.; Borra, G.; Palla, G.; Fiore, C.; Ganucci, D.; Caligiani, A.; Brandolini, V. The species-specific ratios of 2,3-butanediol and acetoin isomers as a tool to evaluate wine yeast performance. *Int. J. Food Microbiol.* **2003**, *86*, 163–168. [CrossRef]
13. Romano, P.; Suzzi, G. Origin and production of acetoin during wine yeast fermentation. *Appl. Environ. Microbiol.* **1996**, *62*, 309.
14. Sadoudi, M.; Tourdot-Maréchal, R.; Rousseaux, S.; Steyer, D.; Gallardo-Chacón, J.J.; Ballester, J.; Vichi, S.; Guérin-Schneider, R.; Caixach, J.; Alexandre, H. Yeast-yeast interactions revealed by aromatic profile analysis of Sauvignon Blanc wine fermented by single or co-culture of non-*Saccharomyces* and *Saccharomyces* yeasts. *Food Microbiol.* **2012**, *32*, 243–253. [CrossRef] [PubMed]
15. Wang, C.; Mas, A.; Esteve-Zarzoso, B. The interaction between *Saccharomyces cerevisiae* and non-*Saccharomyces* yeast during alcoholic fermentation is species and strain specific. *Front. Microbiol.* **2016**, *7*, 502. [CrossRef]
16. Morata, A.; Loira, I.; Vejarano, R.; González, C.; Callejo, M.J.; Suárez-Lepe, J.A. Emerging preservation technologies in grapes for winemaking. *Trends Food Sci. Technol.* **2017**, *67*, 36–43. [CrossRef]
17. Escott, C.; Loira, I.; Morata, A.; Bañuelos, M.A.; Antonio Suárez-Lepe, J. Wine Spoilage Yeasts: Control Strategy. In *Yeast—Industrial Applications*; Morata, A., Loira, I., Eds.; InTech: London, UK, 2017; p. 98.
18. Delfini, C.; Gaia, P.; Schellino, R.; Strano, M.; Pagliara, A.; Ambrò, S. Fermentability of grape must after inhibition with Dimethyl Dicarbonate (DMDC). *J. Agric. Food Chem.* **2002**, *50*, 5605–5611. [CrossRef] [PubMed]
19. Csutak, O.; Vassu, T.; Sarbu, I.; Stoica, I.; Cornea, P. Antagonistic activity of three newly isolated yeast strains from the surface of fruits. *Food Technol. Biotechnol.* **2013**, *51*, 70–77.
20. Janisiewicz, W.J.; Tworkoski, T.J.; Kurtzman, C.P. Biocontrol potential of *Metchnikowia pulcherrima* strains against blue mold of apple. *Phytopathology* **2001**, *91*, 1098–1108. [CrossRef]
21. Kántor, A.; Hutková, J.; Petrová, J.; Hleba, L.; Kačániová, M. Antimicrobial activity of pulcherrimin pigment produced by *Metschnikowia pulcherrima* against various yeast species. *J. Microbiol. Biotechnol. Food Sci.* **2015**, *5*, 282–285. [CrossRef]
22. Oro, L.; Ciani, M.; Comitini, F. Antimicrobial activity of *Metschnikowia pulcherrima* on wine yeasts. *J. Appl. Microbiol.* **2014**, *116*, 1209–1217. [CrossRef] [PubMed]
23. Saravanakumar, D.; Ciavorella, A.; Spadaro, D.; Garibaldi, A.; Gullino, M.L. *Metschnikowia pulcherrima* strain MACH1 outcompetes *Botrytis cinerea*, *Alternaria alternata* and *Penicillium expansum* in apples through iron depletion. *Postharvest Biol. Technol.* **2008**, *49*, 121–128. [CrossRef]
24. Sipiczki, M. *Metschnikowia* strains isolated from botrytized grapes antagonize fungal and bacterial growth by iron depletion. *Appl. Environ. Microbiol.* **2006**, *72*, 6716–6724. [CrossRef]

25. Lopes, C.A.; Sáez, J.S.; Sangorrín, M.P. Differential response of *Pichia guilliermondii* spoilage isolates to biological and physico-chemical factors prevailing in Patagonian wine fermentations. *Can. J. Microbiol.* **2009**, *55*, 801–809. [CrossRef]
26. Sangorrín, M.P.; Lopes, C.A.; Jofré, V.; Querol, A.; Caballero, A.C. Spoilage yeasts from Patagonian cellars: Characterization and potential biocontrol based on killer interactions. *World J. Microbiol. Biotechnol.* **2008**, *24*, 945–953. [CrossRef]
27. Lopes, C.A.; Sangorrín, M.P. Optimization of killer assays for yeast selection protocols. *Rev. Argent. Microbiol.* **2010**, *42*, 298–306.
28. Spadaro, D.; Ciavorella, A.; Dianpeng, Z.; Garibaldi, A.; Gullino, M.L. Effect of culture media and pH on the biomass production and biocontrol efficacy of a *Metschnikowia pulcherrima* strain to be used as a biofungicide for postharvest disease control. *Can. J. Microbiol.* **2010**, *56*, 128–137. [CrossRef]
29. Piano, S.; Neyrotti, V.; Migheli, Q.; Gullino, M.L. Biocontrol capability of *Metschnikowia pulcherrima* against *Botrytis* postharvest rot of apple. *Postharvest Biol. Technol.* **1997**, *11*, 131–140. [CrossRef]
30. Varela, C.; Sengler, F.; Solomon, M.; Curtin, C. Volatile flavour profile of reduced alcohol wines fermented with the non-conventional yeast species *Metschnikowia pulcherrima* and *Saccharomyces uvarum*. *Food Chem.* **2016**, *209*, 57–64. [CrossRef]
31. Zhang, B.-Q.; Shen, J.-Y.; Duan, C.-Q.; Yan, G.-L. Use of indigenous *Hanseniaspora vineae* and *Metschnikowia pulcherrima* co-fermentation with *Saccharomyces cerevisiae* to improve the aroma diversity of Vidal Blanc icewine. *Front. Microbiol.* **2018**, *9*, 2303. [CrossRef]
32. Zott, K.; Thibon, C.; Bely, M.; Lonvaud-Funel, A.; Dubourdieu, D.; Masneuf-Pomarede, I. The grape must non-*Saccharomyces* microbial community: Impact on volatile thiol release. *Int. J. Food Microbiol.* **2011**, *151*, 210–215. [CrossRef]
33. Jolly, N.P.; Augustyn, O.P.H.; Pretorius, I.S. The Role and Use of Non-Saccharomyces Yeasts in Wine Production. Available online: https://pdfs.semanticscholar.org/2c05/c11843cafd3d1ed97dcbefce97964537386a.pdf (accessed on 7 June 2019).
34. Ganga, M.A.; Martínez, C. Effect of wine yeast monoculture practice on the biodiversity of non-*Saccharomyces* yeasts. *J. Appl. Microbiol.* **2004**, *96*, 76–83. [CrossRef]
35. Reid, V.J.; Theron, L.W.; Du Toit, M.; Divol, B. Identification and partial characterization of extracellular aspartic protease genes from *Metschnikowia pulcherrima* IWBT Y1123 and *Candida apicola* IWBT Y1384. *Appl. Environ. Microbiol.* **2012**, *78*, 6838–6849. [CrossRef]
36. Romano, P.; Capece, A.; Jespersen, L. Taxonomic and ecological diversity of food and beverage yeasts. In *Yeasts in Food and Beverages*; Springer: Berlin/Heidelberg, Germany, 2006; pp. 13–53.
37. Mendes Ferreira, A.; Clímaco, M.C.; Mendes Faia, A. The role of non-*Saccharomyces* species in releasing glycosidic bound fraction of grape aroma components-a preliminary study. *J. Appl. Microbiol.* **2001**, *91*, 67–71. [CrossRef]
38. Manzanares, P.; Rojas, V.; Genovés, S.; Vallés, S. A preliminary search for anthocyanin-β-D-glucosidase activity in non-*Saccharomyces* wine yeasts. *Int. J. Food Sci. Technol.* **2000**, *35*, 95–103. [CrossRef]
39. Gunata, Y.Z.; Bayonove, C.L.; Baumes, R.L.; Cordonnier, R.E. The aroma of grapes I. Extraction and determination of free and glycosidically bound fractions of some grape aroma components. *J. Chromatogr. A* **1985**, *331*, 83–90. [CrossRef]
40. Gunata, Z.; Bitteur, S.; Brillouet, J.-M.; Bayonove, C.; Cordonnier, R. Sequential enzymic hydrolysis of potentially aromatic glycosides from grape. *Carbohydr. Res.* **1988**, *184*, 139–149. [CrossRef]
41. Ganga, M.A.; Carriles, P.; Raynal, C.; Heras, J.M.; Ortiz-Julien, A.; Dumont, A. Vincular la *Metschnikowia pulcherrima* y la *Saccharomyces cerevisiae* Para una Máxima Revelación del Aroma en Vinos Blancos. Available online: https://www.lallemandwine.com/wp-content/uploads/2014/10/Flavia-Lee-el-documento.pdf (accessed on 7 June 2019).
42. Gunata, Z.; Brillouet, J.M.; Voirin, S.; Baumes, R.; Cordonnier, R. Purification and some properties of an alpha-L-arabinofuranosidase from *Aspergillus niger*. Action on grape monoterpenyl arabinofuranosylglucosides. *J. Agric. Food Chem.* **1990**, *38*, 772–776. [CrossRef]
43. Quirós, M.; Rojas, V.; Gonzalez, R.; Morales, P. Selection of non-*Saccharomyces* yeast strains for reducing alcohol levels in wine by sugar respiration. *Int. J. Food Microbiol.* **2014**, *181*, 85–91. [CrossRef]

44. Contreras, A.; Hidalgo, C.; Henschke, P.A.; Chambers, P.J.; Curtin, C.; Varela, C. Evaluation of non-*Saccharomyces* yeasts for the reduction of alcohol content in wine. *Appl. Environ. Microbiol.* **2014**, *80*, 1670–1678. [CrossRef]
45. Morales, P.; Rojas, V.; Quirós, M.; Gonzalez, R. The impact of oxygen on the final alcohol content of wine fermented by a mixed starter culture. *Appl. Microbiol. Biotechnol.* **2015**, *99*, 3993–4003. [CrossRef]
46. Varela, J.; Varela, C. Microbiological strategies to produce beer and wine with reduced ethanol concentration. *Curr. Opin. Biotechnol.* **2019**, *56*, 88–96. [CrossRef]
47. Contreras, A.; Curtin, C.; Varela, C. Yeast population dynamics reveal a potential 'collaboration' between *Metschnikowia pulcherrima* and *Saccharomyces uvarum* for the production of reduced alcohol wines during Shiraz fermentation. *Appl. Microbiol. Biotechnol.* **2015**, *99*, 1885–1895. [CrossRef]
48. Mestre Furlani, M.V.; Maturano, Y.P.; Combina, M.; Mercado, L.A.; Toro, M.E.; Vazquez, F. Selection of non-*Saccharomyces* yeasts to be used in grape musts with high alcoholic potential: A strategy to obtain wines with reduced ethanol content. *FEMS Yeast Res.* **2017**, *17*. [CrossRef]
49. Morata, A.; Loira, I.; Suárez Lepe, J.A. Influence of yeasts in wine colour. In *Grape and Wine Biotechnology*; Morata, A., Loira, I., Eds.; InTech: London, UK, 2016; pp. 298–300.
50. Morata, A.; Gómez-Cordovés, M.C.; Suberviola, J.; Bartolomé, B.; Colomo, B.; Suárez, J.A. Adsorption of anthocyanins by yeast cell walls during the fermentation of red wines. *J. Agric. Food Chem.* **2003**, *51*, 4084–4088. [CrossRef]
51. Morata, A.; Gómez-Cordovés, M.C.; Colomo, B.; Suárez, J.A. Cell wall anthocyanin adsorption by different *Saccharomyces* strains during the fermentation of *Vitis vinifera* L. cv Graciano grapes. *Eur. Food Res. Technol.* **2005**, *220*, 341–346. [CrossRef]
52. Caridi, A.; Sidari, R.; Kraková, L.; Kuchta, T.; Pangallo, D. Assessment of color adsorption by yeast using Grape Skin Agar and impact on red wine color. *OENO One* **2015**, *49*, 195–203. [CrossRef]