DOI : https://doi.org/10.58233/1pRdYwlE

The chances for using non-*Saccharomyces* wine yeasts for sustainable winemaking

Maurizio Ciani[1], Laura Moretti[1], Silvia Gattucci[1], Laura Canonico[1]

[1] Università Politecnica delle Marche, via Brecce Bianche, 60100Ancona, Italy

Résumé. Climate changes and the trend towards organic and more sustainable winemaking highlighted the need to use biological methodologies. The reduction in the use of SO_2, the need for the reduction of ethanol content of wines, and the need to reduce or avoid the use of chemical phytosanitary products have prompted the search for alternative practices. In this context, the use of non-*Saccharomyces* wine yeasts is promising for achieving environmental, economic, and health sustainability objectives.
Here, it is presented the various possible uses of non-*Saccharomyces* wine yeasts in the entire winemaking chain, from the vineyard for phytosanitary protection and control of the grape microbiota to biocontrol actions in the pre-fermentation and fermentation phase for the reduction of the use of SO_2, to use in mixed fermentations to reduce the alcohol content of wines, to use in the drying of the grapes for the production of special wines and post-harvest. These uses, as amply demonstrated by the literature, are also associated with an improvement and an increase of complexity of the sensorial analytical profile of the wines.

1. Introduction

Climate changes and the trend towards organic and more sustainable winemaking highlighted the need to use biological methodologies. Global climate change has deeply influenced the vine phenology and the grape composition, resulting in grapes with lower acidity, altered phenolic maturation, and tannin content, increasing sugar concentration [1]. This determined an increase in ethanol content in wine (2% v/v over the past 20 years) and acidity reduction [2].

Another of the most relevant concerns in wine production is the need for the reduction of chemical compounds in the wine production chain. The reduction of SO_2, particularly at the pre-fermentative stage, and the necessity to reduce or eliminate chemical phytosanitary products have prompted the search for alternative practices.

In this context, the use of non-*Saccharomyces* wine yeasts is promising for achieving environmental, economic, and health sustainability objectives.

Here, investigations and applied uses of non-*Saccharomyces* wine yeasts in the whole winemaking chain were reported.

2. Use and potentiality of non-*Saccharomyces* wine yeasts

2.1. Non-*Saccharomyces* as biocontrol agent in Vineyard

The use of microorganisms as natural biological agents was defined as the reduction in pathogen or disease activities through organisms or their molecules. The use of microorganisms with antagonist action against other microorganisms is a strategy for lowering the use of pesticides and boosting food quality and safety.

The addition of microorganisms as bio-protective agents or their antimicrobial products has already been identified as “bio-protection”. Regarding the application of non *Saccharomyces* yeasts in bio-protection strategies against grapevine trunk diseases in the vineyard (the fungal pathogen *Botrytis cinerea* causing bunch sour rot), research has increased significantly in recent years and several yeast species were proposed. Among them, *Aureobasidium pullulans* and *Metschnikowia pulcherrima* were found promising potential biocontrol agents for controlling the development of *B. cinerea* mold. In vineyards trials showed their anti-*B. cinerea* action and could be proposed as a single species or in combination by exploiting the synergistic action of their antagonistic capacities through the rapid colonization of the grapes and persistence on the grape surface [3]. In this regard, preliminary investigations showed a good ability of *M. pulcherrima* strains to colonize the grape surface and persist until harvest time with possible enrichment of must and positive action during the post-harvest stages.

2.2. Non-*Saccharomyces* as biocontrol agent at pre-fermentative stage

In addition to using non-*Saccharomyces* yeasts in vineyard, there is a growing interest in their application in the whole wine production chain as well as during the conservation and maturation phases [4,5]. The interest in biological control is due to the growing attention to the use of sulfites in wine production. In this regard, non *Saccharomyces* yeasts could be a valid and natural alternative to SO_2. Several recent studies have been conducted with selected strains of *M. pulcherrima* and *Torulaspora delbrueckii* at the pre-fermentative stage in the red winemaking process [4,6]. Other studies focused attention on the use of other selected strains of *M. pulcherrima* during the cold clarification stage of the Italian Verdicchio white variety underlining the double role of this yeast as a biocontrol agent and wine aroma enhancer [7].

2.3. Non-*Saccharomyces* as biocontrol agent at fermentation stage

The biocontrol in sequential fermentation of non-*Saccharomyces* yeast is documented by several investigations. Bio-protectant and antioxidant effects of *T. delbrueckii* inoculated at the beginning of the white winemaking process were reported [4]. A biocontrol action of a blend of *T. delbrueckii* and *Metschnikowia pulcherrima,* inoculated at a machine harvester of Cabernet Sauvignon variety, was found compared with a standard addition of SO_2 [8]. A 2-days sequential fermentation of *T. delbrueckii*/*S.cerevisiae* showed control of wild yeasts only slightly lower during the first two days of fermentation if compared with the addition of SO_2. The strain *T. delbrueckii* DiSVA 130 effectively limited the development of wild yeasts demonstrating its effectiveness in protecting must [9].

Table 1. Non-*Saccharomyces* yeast in biocontrol winemaking process.

Non-Saccharomyces species	Environment	Antimicrobial features
Aureobasidium pullulans	Table grape berries	Biocontrol VOC mediated
Aureobasidium pullulans	Grape vine vineyard	Control of Botrytis cinerea
Candida intermedia	Wine environment	Anti-spoilage yeasts (wide spectrum) AMPs mediated
Metschnikowia pulcherrima	Grape vine vineyard	Control of Botrytis cinerea
Metschnikowia pulcherrima	Pre-fermentative stage In sequential fermentation	Biocontrol action
Torulaspora delbrueckii	Pre-fermentative stages In sequential fermentation	Biocontrol action
Metschnikowia pulcherrima	Postharvest Table grapefuits bio-packaging	Anti-mould activity

2.4. Non-*Saccharomyces* yeasts in *Brettanomyces* yeasts control

Several works have been focused on the study of Non-*Saccharomyces* able to counteract the development of *Brettanomyces* spp., a relevant dangerous yeast in winemaking. Two mycocins, Pikt and Kwkt mycocins, produced by *Wickerhamomyces anomalus* (formerly *Pichia anomala*) and *Kluyveromyces wickerhamii*, respectively can counteract *Brettanomyces* yeasts [10]. Cytofluorimetric evaluation showed that both Pikt and Kwkt caused irreversible death of this yeast differently by sulfur dioxide that induced a viable but non-cultivable (VBNC) state of *Brettanomyces* with a consequent recovery of yeasts when fresh medium was replaced [11]. Another mycocin named WA18 produced by a strain of *W. anomalus* is active against *Brettanomyces bruxellensis* is produced by an autochthonous isolated from soil pit and exhibited 99% identity with UDP-glycosyltransferase protein [12]. Another *Pichia membranifaciens* strain showed a killer action producing two mycocins denominated PMKT and PMKT2. The killer activity of *P. membranifaciens* was exploited in winemaking to control *B. bruxellensis* using mixed fermentation with *S. cerevisiae* and *B. bruxellensis* (inoculum ratio of 1:1) *P.membranifaciens* inhibited *B. bruxellensis* growth without any effects on the fermentation activity of *S. cerevisiae*. Other mycocins (CpKT1 and CpKT2) active against *B. bruxellensis* are produced by *Candida pyralidae*. Both mycocins were active and stable in general conditions of the winemaking environment Their use in mixed fermentation in red grape juice containing *B.*

bruxellensis, determined a decrease in spoilage yeast concentration [13]. In addition, strains of *T. delbrueckii* possessing the killer trait and capable of controlling spoilage yeasts have also been found. Td 1 td 2 [14,15]. All these killer yeast show promising potential to counteract *Brettanomyces* yeasts using biological means.

2.5. Non-*Saccharomyces* for ethanol reduction and enhance titratable acidity

There is a growing interest in investigating non *Saccharomyces* wine yeasts in ethanol reduction. Indeed, non *Saccharomyces* yeast generally show a low ethanol yield that could be a potential tool for reducing ethanol in wine. Several works investigated on interspecies and/or intraspecies variability in ethanol yield among non *Saccharomyces* wine yeasts [16,17]. Since most non *Saccharomyce*s yeast are incapable of completing alcoholic fermentation *S. cerevisiae* wine strain should be added in simultaneously or sequentially. In this regard, several works investigated mixed fermentation with *M. pulcherrima*, *T. bacillars*, *T. bombicola*, *Z. rouxii*, *T. delbrueckii*, and *P. kudriavzevii* The regulatory respiro-fermentative metabolism in yeasts might be used as a strategy to reduce the ethanol concentration in wine. In addition to a low ethanol yield, among non-*Saccharomyces* wine yeasts some strains/species showed sugar consumption by respiration (Crabtree negative). Both these approaches have indicated the promising use of non *Saccharomyces* wine yeast to limit ethanol production. Indeed, more recent works using this approach showed promising results. Three strains of *M. pulcherrima*,*T. delbrueckii* and *Z. bailii* sequential fermentations with *S. cerevisiae* under different aeration conditions showed an ethanol reduction of 1.6% v/v, 0.9% v/v and 1.0% v/v for *M. pulcherrima*, *T. delbrueckii* and *Z. bailii* sequential fermentations, respectively with a volatile profile without an excess of acetic acid. [18]. Bench-Top fermentation trials with different aeration conditions using *M. pulcherrima* selected strain with aeration flow of 20 mL/L/min during the first 72 h of fermentation, led to an ethanol reduction of 1.38% (v/v) without any negative feature. Indeed, the concentration of ethyl acetate did not negatively impact while significant fruity and flower compounds were found. [19]. *S. bombicola*/*S. cerevisiae* sequential fermentation under aeration condition determined an ethanol reduction of 1.46% (*v*/*v*). Aeration condition did not negatively affect the analytical profile of sequential fermentation *S. bombicola*/*S. cerevisiae* (volatile acidity and ethyl acetate). On the other hand, these conditions strongly improved the production of glycerol and succinic acid, which positively affect the structure and body of wine [20].

Global climate change is causing a shift in the grapes used for winemaking, leading to increasing sugar content and serious decreases in the acidities of grape juices, particularly those that originate in warm and temperate climates. Some viticultural regions that traditionally have been considered cool climates are beginning to suffer from similar concerns. Another biological alternative, to impact of climate change in winemaking, is the enhancement of titrable acidity.

Among non-*Saccharomyces* yeasts *Lachancea thermotolerans* is the species most investigated and used in winemaking. The main features of *L. thermotolerans* in co-culture or sequential fermentation are pH reduction, enhancement of 2-phenyl ethanol and glycerol. Indeed, undue different conditions as modalities of inoculum, temperature of fermentation, and different grape juices this parameters were constantly enhanced [21]. In a most recent investigation, *L. thermotolerans* strains including commercial strains and wine-related natural isolates showed differences in basic chemical parameters such as lactic acid, malic acid, ethanol concentrations, and the volatile profile, particularly in sequential fermentations highlighting the synergic effect between the species. The specific chemical profiles of these wines were confirmed by the sensory analysis test, which expressed these results at the tasting level as significant increases in the spicy notes and total acidity increases. [22-23].

In conclusion, non *Saccharomyces* yeasts have great potential to be used to mitigate the effect of climate changes in winemaking and to improve the healthiness of wine by avoiding or reducing the use of SO_2. Some uses are already applied by operators but many potential uses still need to be tested and validated to be usefully applied in winemaking.

3. References

1. Jones, G. V., White, M. A., Cooper, O. R., and Storchmann, K. Clim. Change 73, 319–343 (2005).
2. Gonzalez, R., Quirós, M., and Morales, P. Trends Food Sci. Technol. 29, 55–61(2013).
3. Agarbati, A., Canonico, L., Pecci, T., Romanazzi, G., Ciani, M., Comitini, F. Microorganisms 10, 200 (2022)
4. Simonin, S., Roullier-Gall, C., Ballester, J., Schmitt-Kopplin, P., Quintanilla-Casas, B., Vichi, S., Tourdot-Maréchal, R. Front. Microbiol. 11, 1308. (2020)
5. Escribano-Viana, R., González-Arenzana, L., Garijo, P., Fernández, L., López, R., Santamaría, P., Gutiérrez, A.R., Fermentation, 8, 337 (2022)
6. Windholtz, S., Dutilh, L., Lucas, M., Maupeu, J., Vallet-Courbin, A., Farris, L., Masneuf-Pomarède, I. Appl. Sci. 11, 2494. (2021)
7. Canonico, L., Agarbati, A., Galli, E., Comitini, F., Ciani, M. LWT, 181, 114758. (2023)
8. Chacon-Rodriguez, L.; Joseph, C.M.L., Nazaris, B., Coulon, J., Richardson, S., Dycus, D.A. Catal. Discov. Into Pract. 4, 82–90. (2020)
9. Canonico, L., Agarbati, A., Galli, E., Comitini, F., Ciani, M. Foods , 12, 2899 (2023)

10. Comitini, F., de Ingeniis, J.M., Pepe, L., Mannazzu, I., Ciani, M. FEMS Microb. Lett., 238, 235–240. (2004)
11. Oro, L., Ciani, M., Bizzaro, D., Comitini, F. J. Appl. Microbiol. 121, 207–214 (2016),
12. Comitini, F., Agarbati, A., Canonico, L., Galli, E., Ciani, M. Microorganisms , 9, 56 (2020)
13. Mehlomakulu, N.N., Prior, K.J., Setati, M.E., Divol, B. J. Appl. Microbiol. 122, 747–758. (2017)
14. Villalba, M. L., Sáez, J. S., Del Monaco, S., Lopes, C. A., Sangorrín, M. P. In. J. Food Microbiol. 94-100. 217, (2016).
15. Ramírez, M., Velázquez, R., Maqueda, M., López-Piñeiro, A., Ribas, J. C. Front. Microbiol., 6, 983. (2015).
16. Gobbi, M., De Vero, L., Solieri, L. Comitini, F., Oro, L., Giudici, P., Ciani, M. Eur. Food. Res. Technol. 239, 41–48. (2014)
17. Contreras, A., Hidalgo, C., Henschke, P.A., Chambers, P.J., Curtin, C., Varela, C. Appl. Environ. Microbiol. (2014),
18. Canonico, L., Solomon, M., Comitini, F., Ciani, M., Varela, C. Food Microbiol., 84, 103247. (2019).
19. Canonico L, Galli E, Agarbati A, Comitini F, Ciani M. Foods. 10 (5) 1047. (2021)
20. Canonico, L., Comitini, F., Ciani, M. Foods, 8 9), 378. (2019)
21. Gobbi, M., Comitini, F., Domizio, P., Romani, C., Lencioni, L., Mannazzu, I., Ciani, M. Food Microbiol., 33 (2), 271-281. (2013).
22. Morata, A., Loira, I., Tesfaye, W., Bañuelos, M. A., González, C., Suárez Lepe, J. A. Fermentation, 4 (3), 53. (2018)
23. Vicente, J., Kelanne, N., Rodrigo-Burgos, L., Navascués, E., Calderón, F., Santos, A., Benito, S. FEMS Yeast Res., 23, foac062. (2023).