

Editorial

Enological Repercussions of Non-*Saccharomyces* Species in Wine Biotechnology

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The use of non-*Saccharomyces* yeasts in enology has increased since the beginning of the current century because of the potential improvements they can produce in wine sensory quality. Several review articles have described the potential of some non-*Saccharomyces* species [1–3] and the suitable criteria to select them [4,5] according to the effects of the species on wine color, aroma, body or structure. Most non-*Saccharomyces* species have low fermentative power, which makes it necessary to use them in sequential fermentations with *S. cerevisiae* to completely deplete the sugars. Moreover, some of them have slow fermentation kinetics, which is a drawback for a competitive implantation in must containing *S. cerevisiae* indigenous populations. Emerging technologies to control wild indigenous yeasts can facilitate the development, growth and fermentative activity of the inoculated non-*Saccharomyces* yeasts and, therefore, the suitable expression of their metabolic properties [6]. This special issue is focused on the description and review of several non-*Saccharomyces* species with great potential in wine biotechnology, some of which are frequently used at the winery scale, but also produced industrially as dried yeast or liquid inoculant [7].

Wine acidity, especially the pH, is a key parameter in wine that controls microbial development and chemical stability. Traditional pH control is driven by acidification processes with tartaric acid or modern ion exchanger techniques, which unfortunately affect sensory quality. The biological modulation of wine acidity can be done efficiently by several non-*Saccharomyces* species, by the production of lactic acid by *Lachancea thermotolerans* or succinic acid by *Candida stellata*, the demalication by *Schizosaccharomyces pombe* or *Pichia kudriavzevii*, and the control of volatile acidity in sequential fermentations with *Torulaspora delbrueckii* or *Zygosaccharomyces florentinus* highlight the possibilities of non-*Saccharomyces* in the improvement of wine acidity [8].

Biological acidification by *L. thermotolerans* is a powerful tool to control pH in warm areas [9]. The production of acidity is performed from sugars and the product lactic acid is a stable metabolite during winemaking but also through stabilization and aging. The formation of several metabolites with sensory repercussions has also been described in this species. Acidification by *L. thermotolerans* is a natural biotechnology that helps to keep lower and more effective levels of molecular and free SO₂. Currently, in our laboratory we have selected strains of this species able to ferment at more than 12% potential alcohol, which opens the door to single fermentations with single inoculums of *L. thermotolerans*.

Wine deacidification by metabolization of malic acid is an essential step in red winemaking. This acid is unstable during stabilization and aging, and can produce microbial hazes if not eliminated previously. Usually, malic acid is transformed into lactic acid by malolactic fermentation produced by lactic acid bacteria, mainly *Oenococcus oeni*, due to the specific composition of wine. Alternatively, *S. pombe* is able to metabolize malic acid by the maloalcoholic fermentation pathway. The advantages are the fast and efficient degradation of malic acid and at the same time *S. pombe* can produce the alcoholic fermentation. Moreover, its use reduces the formation of biogenic amines. Also, the peculiar metabolism of *S. pombe* facilitates the formation of vitisin A pyranoanthocyanin pigments, with positive effects on color stability [10].

Among the pioneer species used in enology is *T. delbrueckii*, with medium fermentative power, some strains reach 9%–10% in alcohol with a high fermentation purity. The production of acetic esters and other specific aromas makes this yeast a key option to improve wine aroma, but it also has interesting effects on the body and structure [11]. Recently, it has been used in sparkling wines to make more complex base wines, whilst also increasing the structure during bottle aging [12].

The production of acetic esters is an interesting strategy to improve a wine's aromatic profile. The use of *Wickerhamomyces anomalus* helps to increase the contents of several esters, specifically 2-phenyl-ethyl acetate, with positive floral profiles [13]. The main drawback of this species is the high production of acetic acid, which can be partially controlled with suitable strain selection, but also through its use in sequential fermentation with *S. cerevisiae*. Apiculate species, such as the *Hanseniaspora/Kloeckera* genera, are also described as strong producers of acetate esters, and many species enhance the formation of 2-phenyl-ethyl acetate; some also produce benzenoids or nor-isoprenoids. Moreover, they tend to have an interesting effect on structure by producing full bodied wines [14]. Some of these species, as well as *Metschnikowia pulcherrima* and *C. stellata*, are able to release extracellular hydrolytic enzymes, such as β -glucosidases or c-lyases, that help improve the varietal aroma by releasing free terpenes or thiols [15,16]. A wide pool of enzymatic activities can also be found in saprophytic *Aureobasidium pullulans*, several of these enzymes can be purified with useful applications in enology [17]. *A. pullulans* is a typical yeast-fungus that can be found in the indigenous microbiota of the berry together with the apiculate genera *Hanseniaspora/Kloeckera*.

Spoilage yeasts such as *Zygosaccharomyces rouxii*, *Saccharomyces ludwigii* or *Brettanomyces bruxellensis* may be difficult to handle at specific winemaking stages. Usually, the main concern of the enologist is their control and elimination from musts and wines, but also the analysis of their populations and their main marker metabolites. However, these non-*Saccharomyces* species sometimes have interesting applications in fermentative industries. *Zygosaccharomyces rouxii* is a frequent osmophilic spoilage species that causes re-fermentations in sweet wines and other drinks, such as fruit juices and soft beverages. Its control can be done using additives as DMDC, emerging antimicrobials as LfcinB, or cold pasteurization processes as DBD, US, UHPH or PEFs [18]. *Saccharomyces ludwigii* is a strong fermenting yeast able to completely finish grape sugars; it also shows a strong resistance to high SO₂ levels. Some interesting applications are now being described, such as the use of this species in the reduction of the alcoholic degree of beers or in the production of ciders. In enology, the production of off-flavors reduces a lot the potential use of *S. ludwigii* in wine fermentation. The control measures used to reduce its prevalence in wines are the use of emerging physical technologies, chemical additives such as DMDC, but also natural products such as chitosan or biological control with killer yeasts [19]. The use of biological control with yeasts able to produce antimicrobial peptides is a novelty in the elimination of *Brettanomyces* spp. [20]. This spoilage yeast degrades the sensory quality of the wine as it develops during barrel aging, usually affecting more expensive wines by producing several unpleasant molecules [21]. Conventional control is based on the use of SO₂ and hygiene measures, however both parameters are difficult to control and maintain during long periods in difficult materials such as barrel wood. The use of *C. intermedia* as a selective bio-controller is a natural way to reduce the damages produced by *Brettanomyces*. Bio-protection and biological management of spoilage and undesired yeast can be also done by using *M. pulcherrima*, the production of the pigment pulcherrimin and their effect on iron chelation helps to eliminate competitive yeasts in grapes or at the beginning of fermentation [15].

If the twentieth century saw the explosion of *S. cerevisiae* applications, non-*Saccharomyces* yeasts open up a world of new biotechnologies in the twenty-first century, including improved fermentations, with more complex and differentiated sensory profiles in wines, bioprotection applications, enzymatic activities, acidity modulation, improvement of aging processes, reduction of toxic molecules and additives, and many other possibilities to discover. Some of these potentials contribute to the adaptation of wine to regions and terroirs, even to the ecological changes produced by global warming.

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