



Microbial terroir and food innovation: The case of yeast biodiversity in wine



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ABSTRACT

Saccharomyces and non-*Saccharomyces* represents a heterogeneous class in the grape/must/wine environments including several yeast genera (e.g., *Saccharomyces*, *Hanseniaspora*, *Pichia*, *Candida*, *Metschnikowia*, *Kluyveromyces*, *Zygosaccharomyces*, *Torulaspora*, *Dekkera* and *Schizosaccharomyces*) and species. Since, each species may differently contribute to the improvement/depreciation of wine qualities, it appears clear the reason why species belong to non-*Saccharomyces* are also considered a biotechnological resource in wine fermentation. Here, we briefly review the oenological significance of this specific part of microbiota associated with grapes/musts/wine. Moreover, the diversity of cultivable non-*Saccharomyces* genera and their contribute to typical wines fermentations will be discussed.

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1. Wine and microbial resources

Wine is the result of a complex biochemical process, that starts with grape harvesting, continues with the alcoholic (AF) and malolactic (MLF) fermentations, wine aging and bottling (Romano et al., 2003). From the microbiological point of view, winemaking involves two main phases, the so-called alcoholic fermentation and malolactic fermentation, and an heterogeneous microbiota composed by different microorganism (e.g., yeast, bacteria and filamentous fungi) with considerable possible interaction among them (Fleet, 2007; Fugelsang and Edwards, 2007).

Although of the entire wine microflora contribute to the wine chemistry, yeasts detain a predominant role, since they promote the AF, a composite biochemical process where grape sugars are transformed into ethanol and carbon dioxide, producing at the same time a large number of additional by-products (Fleet and Heard, 1993; Fugelsang, 1997). In modern winemaking, AF is typically carried out using biomasses of selected *Saccharomyces cerevisiae* strains (microbial starter). However, traditional fermentation occur through a spontaneous process performed by the sequential action of different yeast species/strains, naturally present on the berries grapes or in the winery and selected in reason of ecological determinants and of technological parameters (Ciani et al., 2009). The secondary fermentation in wine is the MLF. Rather than a proper 'fermentation', MFL is a bacterial driven decarboxylation of L-malic acid into L-lactic acid and CO₂ operated by lactic acid bacteria (LAB), mostly belong to *Oenococcus oeni* and

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Lactobacillus plantarum species (Lerm et al., 2010). During MLF, LAB consume the residual carbon sources making them unavailable for spoilage microbes, that could affect the organoleptic properties of wine. Therefore, MLF usually enhances wine sourness and favors microbial stability.

In modern wineries is well diffuse the use of commercial starter culture to steer fermentations. Nevertheless, a debate is still open about the use of a commercial starter, since they often lack of some advantageous enological traits, which are present when the spontaneous fermentation is ruled by indigenous populations (Fleet and Heard, 1993).

The main objection to the use of selected starter cultures is the standardization of wine quality, a characteristic useful for table wine, but undesirable for fine wines, while spontaneous fermentations often suffer of fermentation sucks and arrests, with concomitant production of undesired metabolites (Spano et al., 2010; Capozzi et al., 2011).

Currently, there is rising request for autochthonous yeast starters, which are potentially adapted to a definite grape must and reflect the biodiversity of a particular area, which support the idea that indigenous yeast strains can be associated with a “terroir” (Bokulich et al., 2014; Gilbert et al., 2014).

The preservation of spontaneous microflora is essential to obtain the typical flavor and aroma of wines deriving from different grape varieties (Pretorius, 2000). For that reason, the exploitation of indigenous strains biodiversity has great importance for the characterization and selection of strains with peculiar phenotypes (Capece et al., 2010; Grieco et al., 2011; Tristezza et al., 2014). Furthermore, during the last years, an increasing demand of “organic wine” (produced according to the European Commission Implementing Regulation (EU) No 203/2012 of March 8th 2012) was observed as well as several studies aim to develop new organic-free procedures and able to meet organic perspective with safety of food fermentations (Suzzi et al., 2012; Mendoza et al., 2011; Settanni et al., 2012; Capece et al., 2010). Moreover, the employment of a mixed non-*Saccharomyces/Saccharomyces* starter, able to mimic the natural biodiversity, could be a valid alternative to spontaneous fermentations, since the multi-starter ability to increase the organoleptic properties of wine and to minimize the microbial spoilage.

In order to testify the efforts in the characterization and selection of autochthonous microbial resources for the production of typical wines, in Table 1, we reported the studies conducted in a specific region (Apulia, Southern Italy).

The natural biodiversity of autochthonous microbial resources of typical Apulian grape cultivars, has been widely investigated either in term of yeasts (De Benedictis et al., 2011; Tristezza et al., 2012, 2013, 2014; Garofalo et al., 2015a,b), or lactic acid bacteria (Cappello et al., 2008; Capozzi et al., 2010, 2012, 2014; Lamontanara et al., 2014, 2015; Garofalo et al., 2015b) and spoilage yeasts, such as *Brettanomyces bruxellensis* (Di Toro et al., 2015). Therefore, if we imagine that similar efforts are conducted worldwide in several regions of oenological interest, it appears clear the scientific and economic relevance of this phenomena.

2. Evolution of yeasts during spontaneous fermentation

Grape must contains several yeast species, mainly belonging to the genera *Saccharomyces*, *Pichia*, *Candida*, *Hanseniaspora*, *Metschnikowia* and *Kluyveromyces* (Romano et al., 2003). Sometimes, other yeast species, such as *Torulaspora*, *Saccharomycodes*, *Dekkera*, *Zygosaccharomyces* and *Schizosaccharomyces*, are also observed (Fleet, 2003, 2008; Romano et al., 2003). Non-*Saccharomyces* species start the AF process (especially yeast belonged to the genera *Hanseniaspora*, *Candida*, *Pichia* and *Metschnikowia*), but they are quickly replaced by *S. cerevisiae*, that lead AF until its end, often being the only species detectable at this last stage (Fleet and Heard, 1993; Fleet, 2003).

Several studies undertaken in different countries attributed an important contribution of non-*Saccharomyces* species to yeast growth dynamics during wine fermentations (Pramateftaki et al., 2000; Jolly et al., 2003; Combina et al., 2005a,b; Zott et al., 2011). Hence, non-*Saccharomyces* yeast species supply a factor of diversity that requires specific studies to avoid any negative consequences, and to exploit their beneficial contributions (Jolly et al., 2003). In addition, during the last years, the improvement and application of molecular approaches for the analysis of yeast populations, shown that, together with species variability, spontaneous fermentation is characterized by a significant intraspecific biodiversity (Cocolin et al., 2000), as well as by an high genetic polymorphism observed in the population of *S. cerevisiae* present during spontaneous fermentation. In other words, the population of yeasts correlated to spontaneous fermentations consists of genotypically different strains, usually with different phenotypic properties and, therefore, potentially capable of influencing, in proportion to their relative abundance, the aromatic properties of the final wine product (Romano et al., 2003). Generally, only few *S. cerevisiae* strains are able to dominate the final phases of the process (Vincenzini et al., 2005). Some predominant *S. cerevisiae* strains, recovered from spontaneous fermentation in the same winery, could occur over year, assuming that might be some correlation between strain and winery environment. Additionally, some *S. cerevisiae* strains isolated from different wineries located in the same region could be very similar, highlighting a correlation between strains and enological region (Vigentini et al., 2015).

3. Influence of yeast on wine: some ‘snapshots’ on chemical diversity

The wine organoleptic properties are due to several compounds, usually part of the yeast metabolism, such as higher alcohols, organic acids, esters, aldehydes, fatty acids, and sulfur compounds. By-products arising from yeast metabolism, directly affect wine quality. Moreover, the ratio of non-*Saccharomyces/Saccharomyces* yeasts determines the organoleptic properties of the final product. Several studies on mixed starter cultures are carried out and wines produced differ significantly, concerning both their chemical composition and sensory characteristics (Fleet and Heard, 1993;

Table 1
Studies on autochthonous microbial resources for the oenological sector in Apulian region, Southern Italy.

	References
Yeast (non- <i>Saccharomyces</i> and <i>Saccharomyces</i> spp.)	De Benedictis et al. (2011), Tristezza et al. (2012, 2013, 2014), Garofalo et al. (2015b)
Lactic acid bacteria	Cappello et al. (2008), Capozzi et al. (2010, 2012, 2014), Lamontanara et al. (2015), Garofalo et al. (2015a)
Interaction between <i>Saccharomyces cerevisiae</i> and <i>Oenococcus oeni</i>	Garofalo et al. (2015a)
Spoilage yeast	Di Toro et al. (2014)

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