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Changes in the volatile profile of Pinot noir wines caused by Patagonian *Lactobacillus plantarum* and *Oenococcus oeni* strains

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ABSTRACT

The ability of Patagonian *L. plantarum* and *O. oeni* strains to change the volatile profile of a sterile Pinot noir wine was studied through fermentation assays, at laboratory scale. Two strains of each LAB species were selected based on data regarding to their ability to survive in wine and to consume L-malic acid. Both *O. oeni* strains but only one *L. plantarum* (UNQLp 11) strain were able to remain viable, consuming L-malic acid through the fermentation assay with a concomitant increase of L-lactic acid. The volatile profile of Pinot noir wine, before and after LAB inoculation, was measured by using HS-SPME gas chromatography technique. This analysis showed that alcohols were the main volatile compounds after alcoholic fermentation and that after fermentation with the selected LAB strains, a decrease in the volatile alcohols concentration and an increase in the volatile esters concentration could be observed. The *O. oeni* UNQOe 73.2 strain produced the most notable change in the volatile profile, with the production of some important odorant esters at higher concentration, compared to *O. oeni* UNQOe 31b strain. Although, *L. plantarum* UNQLp 11 strain showed a better performance in the consumption of L-malic acid, this strain had a low capacity to modify the volatile compounds profile after incubation in red wine. The results found in the present work showed that different strains selected as potential malolactic starters could have different behavior when are incubated in real wine. Although *L. plantarum* UNQLp 11 strain showed a good consumption of L-malic acid, the *O. oeni* UNQOe 73.2 strain exhibited superior capacity to improve the flavor of wine due to its esterase activity that produce an increase of fruity and creamy odorants.

1. Introduction

Oenococcus oeni is the major bacterial species found in wines during spontaneous malolactic fermentation (MLF) due to high tolerance to harsh wine conditions (low pH, high ethanol and SO_2 concentrations, low nutrients, and low temperatures) (Wibowo, Eschenbruch, Davis, Fleet, & Lee, 1985). However, *O. oeni* can also be detected with other LAB, mainly *Lactobacillus* spp., and in particular *Lactobacillus plantarum* species (Lonvaud-Funel, 1999, Lerm, Engelbrecht, & du Toit, 2011, Bravo-Ferrada et al., 2013, Valdés La Hens, Bravo-Ferrada, Delfederico, Caballero, & Semorile, 2015,). In the last four decades, the use of malolactic starter cultures has become widespread to control MLF process, and several starter of *O. oeni* and few of *L. plantarum* strains are commercially available (Lerm et al., 2011). MLF generally occurs after alcoholic fermentation (AF) produced by yeast, and it consists in the conversion of L-malic acid to L-lactic acid, resulting in a decrease of titrable acidity, and a small increase in the pH. MLF also leads to enhanced microbial stability and is usually believed to improve the complexity of wine aroma (Cappello, Zapparoli, Logrieco, & Bartowsky, 2017; Iorizzo et al., 2016; Liu, 2002). Several organic acids, in addition to L-malic acid, could be metabolized by LAB during MLF, such as, acetic, citric and tartaric acids. The balance of organic acids has a strong impact on wine taste, being the total consumption of L-malic acid the premise to reduce acidity and astringency of wines (Volschenk, van Vuuren, & Viljoen-Bloom, 2006). On the other hand, some strains of *O. oeni* and *L. plantarum* are able to produce other enzymatic reactions that modify the wine aroma profile (Cappello et al., 2017; Tristezza et al., 2016). Although the presence of a broad range of enzymes in wine LAB have been documented (glycosidases, esterases, phenolic acid decarboxylases, citrate lyases) (Liu, 2002; Ugliano, Genovese, & Moio,

Abbreviations: LAB, lactic acid bacteria; MLF, malolactic fermentation; AF, alcoholic fermentations; MAC, malic acid consumption; HS-SPME, headspace solid phase microextraction

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2003, Matthews et al., 2004, Grimaldi, Bartowsky, & Jiranek, 2005a, 2005b), information on the role of these bacterial enzyme activities including their potential use in winemaking is still limited (Cappello et al., 2017). Glycosidase activities that can affect wine aroma have been detected in wine strains of *Oenococcus*, *Lactobacillus* and *Pediococcus* (Grimaldi et al., 2005a, 2005b). Numerous potential glycosidases have been identified by genome sequence analysis of *O. oeni* strains (Borneman, Bartowsky, McCarthy, & Chambers, 2010), and a gene coding for a β-glucosidase enzyme has been detected in several LAB strains (Spano et al., 2005). The first study on the expression of an *O. oeni* β-glucosidase gene (*hgl*) under winemaking conditions was carried out by Olguín, Alegret, Bordons, and Reguant (2011), highlighting that the factors that influence the gene expression are ethanol concentration, wine matrix, and also the strain involved. On the other hand, changes in ester concentrations have a potential to influence wine quality (Sumby, Grbin, & Jiranek, 2010; Swiegers, Bartowsky, Henschke, & Pretorius, 2005), being responsible for the desirable fruity aroma of young wines. Recent studies demonstrated that wine LAB exhibit two different ester-synthesizing activities that may increase the ethyl ester content thus modulating the fruity-berry character of red wines (Costello, Siebert, Solomon, & Bartowsky, 2013).

In wine, the citrate utilization leads to the production of compounds such as diacetyl, acetoin, butanediol, and acetate which are important contributors to wine aroma and complexity (Bartowsky, E. J.& Henschke, P. A., 2004; Olguín, Bordons, & Reguant, 2009). Citrate lyase is a key enzyme of citrate metabolism since it cleaves citrate into acetate and oxaloacetate, and this latter is decarboxylate to pyruvate, leading onto the formation of diacetyl, which can be further metabolized through to 2,3-butanediol. The organoleptic impact of diacetyl in wine has been debated for many years. Wine tasters generally agree that diacetyl content must not exceed 5–6 mg/L (Davis, Wibowo, Eschenbruch, Lee, & Fleet, 1985), although it depends on the characteristics of each wine (Martineau & Henick-Kling, 1995). Below that level it is considered to contribute to the wine's bouquet, while higher concentrations have a negative impact. For these reasons, the gene encoding the citrate lyase enzyme (*citE*) has been investigated in various wine LAB species (Mills, Rawsthorne, Parker, Tamir, & Makarova, 2005).

Other important volatile compounds found in wines are phenols. The production of volatile phenols in wine are usually associated with *Dekkera bruxellensis* spoilage (Ribéreau-Gayon, Dubourdieu, Donèche, & Lonvaud-Funel, 2006). However, some *Lactobacillus* species have also been shown to be able to produce these compounds, but in lower quantities (Couto, Campos, Figueiredo, & Hogg, 2006). The conversion of phenolic compounds to vanillin (a very powerful aroma compound) by wine LAB has been demonstrated for *Lactobacillus* spp. by Bloem, Bertrand, Lonvaud-Funel, and de Revel (2007). One of the enzymes involved in the production of these compounds is the phenolic acid decarboxylase (PAD), and the presence of a gene encoding for PAD (*pad* gene) in *L. plantarum* and *O. oeni* was reported by several authors (Mtshali, Divol, van Rensburg, & du Toit, 2010, Mtshali, 2011, Lerm et al., 2011).

Argentinean North Patagonia is one of the southernmost wine-growing regions of the world that has optimal agro-ecological conditions for high quality viticulture, in which the Pinot noir varietal has found the optimal conditions to express its full oenological potential (Crisóstomo, 2007). Although the flavor of Pinot noir wine could vary among wine regions, and according to winemaking practices, in general it has a fruity and spicy bouquet (Feng, Skinkis, & Qian, 2017; Girard, Kopp, Reynolds, & Cliff, 1997; Guinard & Cliff, 1987). In Patagonian red wines, MLF occurs spontaneously and randomly, mainly by action of native *L. plantarum* and *O. oeni* strains (Valdés La Hens et al., 2015). In order to avoid delay and spoilage during this process, the use of malolactic starter cultures is an option. However, the commercial cultures are formulated with strains from other wine-growing regions and their use could negatively affect the properties of wine *terroir* (Bokulich, Thorngate, Richardson, & Mills, 2014; González-Arenzana, López, Santamaría, Tenorio, & López-Alfaro, 2012). The selection of autochthonous strains, best adapted to regional winemaking conditions, is desirable. In previous works, we have isolated and characterized, several strains of *L. plantarum* and *O. oeni* from Patagonian Pinot noir wines. Studies of tolerance to wine stress factors in wine-like medium or in sterile wine, as well as the existence of some enzymatic activities and the screening of aroma related genes, allowed us to select the best candidate strains to formulate regional starter cultures for MLF (Bravo-Ferrada et al., 2013; Bravo-Ferrada et al., 2016; Brizuela et al., 2017; Valdés La Hens et al., 2015). The presence of a β-glucosidase gene, and β-glucosidase activity was verified in selected *L. plantarum* and *O. oeni* strains (Bravo-Ferrada et al., 2013; Brizuela et al., 2017). A putative *pad* gene was also analyzed in some Patagonian *O. oeni* and *L. plantarum* strains (Brizuela et al., 2017), although this gene was only found in *L. plantarum* strains. The existence of a citrate lyase complex gene was also screened and reported (Brizuela et al., 2017). This gene was found in all Patagonian *O. oeni* strains analyzed, but only in some *L. plantarum* strains. However, the potential of these Patagonian LAB strains to produce changes in the profile of compounds related to aroma and flavor of regional red wines has not been studied yet.

With this background, the aim of this work was to investigate the ability of previously selected strains of *L. plantarum* and *O. oeni* to modify the volatile compounds profile after inoculation in a sterile Pinot noir (Bravo-Ferrada et al., 2013; Bravo-Ferrada et al., 2016; Bravo-Ferrada, Tymczyszyn, Gómez-Zavaglia, & Semorile, 2014, Brizuela et al., 2017). For this purpose, changes in wine volatile compounds (alcohols, esters, terpenoids, etc.), and in the concentration of some organic acids (mainly L-malic acid) were analyzed before and after inoculation of the sterile wine with these strains.

2. Materials and methods

2.1. Cell acclimation

Bacterial cells in early stationary phase (approximately 10^9 CFU/mL) were collected by centrifugation at 5000 rpm for 10 min and suspended in the same volume of a modified acclimation medium (50 g/L MRS, 40 g/L D(−) fructose, 20 g/L D (−) glucose, 4 g/L L-malate, 1 g/L Tween 80, and 0.1 mg/L pyridoxine, adjusted to pH 4.6) supplemented with 6% v/v ethanol (Bravo-Ferrada et al., 2014).Cultures were incubated during 48 h at 21 °C according to Brizuela et al., 2017.

2.2. Fermentation assays

Two strains of each LAB species (*Lactobacillus plantarum* UNQLp 11, *Lactobacillus plantarum* UNQLp 155, *Oenococcus oeni* UNQOe 31b, *Oenococcus oeni* UNQOe 73.2) were selected for carrying out fermentation assays, at laboratory scale, in sterile Pinot noir wine (at final AF stage). These strains were isolated from Patagonian Pinot noir wines (vintages 2008 and 2014) and selected according to their oenological properties (Bravo-Ferrada et al., 2016, 2013; Brizuela et al., 2017). A volume of 400 mL of wine (14.5% v/v ethanol, pH 3.82, < 2.00 g/L residual sugars, 2 g/L-malic acid, 96 mg/L total SO_2, total acidity of 3.98 g/L) was sterilized by filtration through 0.2 μm pore size (Sartorius Stedim Biotech GmbH, Göttingen, Germany). Acclimated cells were harvested by centrifugation and inoculated (~5×10^7 CFU/mL) in 80 mL of wine. Incubations were performed at 21 °C during 20 days, without shaking.

2.3. Cell viability and L-malic acid consumption

Viable cells were determined by plating on MRS or MLO agar, as appropriate, sampled at days 0, 5, 10, 15 and 20. Remaining L-malic acid was measured with an L-malic acid enzymatic kit (L-Malic Acid Enology enzymatic kit, BioSystems SA, Barcelona, Spain).