International Journal of Food Microbiology 285 (2018) 18–26

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Kinetic and metabolic behaviour of the pectinolytic strain *Aureobasidium pullulans* GM-R-22 during pre-fermentative cold maceration and its effect on red wine quality

María Gabriela Merín*, Vilma Inés Morata de Ambrosini*

Faculty of Science Applied to Industry, National University of Cuyo, Bernardo de Irigoyen 375, 5600 San Rafael, Mendoza, Argentina
National Scientific and Technical Research Council (CONICET), Sede Central, Buenos Aires, Argentina

ARTICLE INFO

Keywords:
Aureobasidium pullulans
Cold-active pectinases
Malbec wine
Pre-fermentative cold maceration
Wine clarification
Sensory profile

ABSTRACT

Pectinolytic yeasts can be applied to winemaking with the purpose of improving sensory and technological properties of wine because of their enzymes secreted during vinification. In this work, the autochthonous yeast-like pectinolytic strain from D.O. San Rafael viticulture region, *Aureobasidium pullulans* GM-R-22, was used in co-culture with *Saccharomyces cerevisiae* IOC 18-2007 in microvinification trials with Malbec must applying pre-fermentative cold maceration (PCM). *A. pullulans* remained viable during PCM and *S. cerevisiae* growth and fermentative kinetics were not affected in mixed culture with respect to pure *S. cerevisiae* culture. High pectinolytic activity (9.13 U/mg) was detected in mixed *A. pullulans* vinification during PCM, in which conditions of low temperature (8 °C), low pH (3.8) and high sugar concentration (250.6 g/L) governed. Mixed *A. pullulans* wine showed enhanced colour compared with pure *S. cerevisiae* wine, characterised by higher colour index and percentage of red colour, lower tonality and percentage of yellow colour, and negative values of b^* and h^* indicating more bluish and purplish tonalities. Moreover, filtration time and turbidity diminished by a 40% in mixed *A. pullulans* wine. The presence of GM-R-22 strain improved the production of desirable volatile compounds, such as esters and norisoprenoids, which displayed the maximum odour activity values (OAVs), whereas this strain reduced the total content of higher alcohols when compared to pure *S. cerevisiae* fermentation. Sensory analysis indicated that *A. pullulans* impacted on wine highlighting the violet hue, plum jam aroma, body and equilibrium that are distinctive features of Malbec variety. *A. pullulans* GM-R-22 seems to be promising for applying to low-temperature red winemaking as an adjunct culture to *S. cerevisiae* to improve the wine quality and vinification process.

1. Introduction

Argentina is the eighth largest wine producer in the world (OIV, 2016), and Malbec (*Vitis vinifera* L.) variety, originated in France, has well adapted to the soil and dry climate of this region becoming the emblematic cultivar of Argentinean viticulture production (INV, 2016). “San Rafael” D.O., a Western Argentinean wine-making region, presents a special microclimate that contributes to distinguish its vineyards and allows the production of some of Argentina's more highly rated Malbec wines, recognised worldwide.

Wine is the result of a complex process involving grape must, microorganisms and winemaking practices. Among microorganisms, yeasts (mainly *Saccharomyces cerevisiae*) are dominating because of their role in driving the alcoholic fermentation. The use of enzymes, especially pectinases, is a traditional oenological practice applied for improving technological process and wine quality (Belda et al., 2016; Cabeza et al., 2009; Piemolini-Barreto et al., 2014; Revilla and González-San José, 2003). Pectinases can help to attain higher juice yield and easier pressing, to enhance clarification and filterability of wine, to release more colour and flavour compounds entrapped in grape skins, thereby facilitating the winemaking process and making a positive contribution to wine sensory properties (Belda et al., 2016; Martín and Morata de Ambrosini, 2014). The addition of commercial pectinases, mainly produced by filamentous fungi, can be costly for oenological industry. In this sense, pectinases of indigenous yeasts have attracted attention from numerous research groups globally as an alternative to commercial pectinases (Belda et al., 2016; Maturano et al., 2012; Merín et al., 2011, 2015). It has been reported that at least 75% of oenological *S. cerevisiae* strains presented pectinolytic activity (Blanco et al., 1994); nevertheless, several studies have detected limited

* Corresponding authors at: Faculty of Science Applied to Industry, National University of Cuyo, Bernardo de Irigoyen 375, 5600 San Rafael, Mendoza, Argentina.
E-mail addresses: mgmerin@fcai.uncu.edu.ar (M.G. Merín), vmorata@fcai.uncu.edu.ar (V.I. Morata de Ambrosini).

https://doi.org/10.1016/j.ijfoodmicro.2018.07.003
Received 22 February 2018; Received in revised form 1 July 2018; Accepted 3 July 2018
Available online 05 July 2018

activity levels in this species (Blanco et al., 1997; Fernández-González et al., 2005; Merín et al., 2014; Merín and Morata de Ambrosini, 2015). Recently, there has been increasing interest in the application of non-*Saccharomyces* wine yeasts, since they can produce substantial sensory complexity in wines (Maturano et al., 2015; Mendoza et al., 2011; Rodríguez et al., 2010); however, the ability of these yeasts to secrete pectinases needs to be thoroughly studied.

Nowadays, winemakers are to introduce novel winemaking practices to enhance the quality of their products. One of the most used techniques for red wine production is pre-fermentative cold maceration (PCM) (Zott et al., 2008). This technique is based on the contact of skins, seeds and other solids with the must in a non-alcoholic setting, in order to favour the extraction of water-soluble compounds such as anthocyanins and aroma precursors (Sacchi et al., 2005). Considering that PCM consists of keeping the must at a low temperature for a certain time, cold-active pectinases might be used to intensify this process, supposing potential advantages such as their functionality during this low-temperature stage that could enhance even more the colour and flavour stability of wines.

Likewise, the wine industry is currently demanding new yeast strains to innovate and improve wine sensory features. In previous reports, we isolated and selected the strain *Aureobasidium pullulans* GM-R-22, an indigenous pectinolytic yeast-like organism able to display cold-active pectinase activity under wine-like conditions, and concluded that it has potential to be applied to the winemaking process (Merín et al., 2011; Merín and Morata de Ambrosini, 2015). Nevertheless, more specific information on the extent of the contribution of *A. pullulans*, a non-conventional species in winemaking, is required. Within this context, the increase in the knowledge about the physiological properties and the metabolic determinants of non-*Saccharomyces* yeasts is the only way to achieve their exploitation in the oenological industry (Belda et al., 2016).

This study examines the behaviour of *A. pullulans* GM-R-22 under microvinification conditions, analysing its growth and pectinase production during PCM, as well as its performance under co-culture with a commercial fermentative strain of *S. cerevisiae* during alcoholic fermentation of Malbec red must. Furthermore, the influence of the pectinolytic strain on chromatic and technological properties, and the aromatic profile and sensory characteristics of the resulting wines were evaluated.

2. Materials and methods

2.1. Microorganisms and growth conditions

Aureobasidium pullulans GM-R-22, previously isolated from the viticulture region D.O. (Denomination of Origin) San Rafael (Mendoza, Argentina), identified and selected on the basis of its ability to produce cold-active pectinases under oenological conditions (Merín et al., 2011; Merín and Morata de Ambrosini, 2015), was used throughout the study.

Saccharomyces cerevisiae IOC 18-2007 (Institut Œnologique de Champagne, France), commercial active dry yeast, was utilised as fermentation starter culture.

The pectinolytic strain is conserved in the Microbial Collection of the Laboratory of Biotechnology (FCAI-UNCuyo) of the SCCM-AAM (Argentine Association of Microbiology) under accession number CCBio-FCAI 020-R22. It was propagated in YPD (containing in g/L: yeast extract 10, peptone 20, glucose 20, pH 4.0) broth at 25 °C for 48 h at least three times prior to experimental use.

2.2. Preparation of starter cultures

Erlenmeyer flasks (100 mL) containing 50 mL of pasteurised grape juice, previously diluted 1:2 with sterile distilled water and adjusted to pH 4.0, were inoculated with 1 mL of culture in exponential phase of *A. pullulans* GM-R-22. Cultures were incubated at 25 °C during 48 h.

Commercial *S. cerevisiae* strain was inoculated at 200 mg/L of active dry yeast, prepared according to the supplier's specifications.

2.3. Preparation of the grape must

Must from *Vitis vinifera* L. cv Malbec, the emblematic red variety of Argentina, obtained from a winery of Rama Caída district (34.66° South latitude and 68.38° West longitude), in D.O. San Rafael wine region, during 2010 vintage, was used to carry out the microvinification trials. The grape must (reducing sugar 250.6 g/L, assimilable nitrogen 180 mg/L, titratable acidity 3.6 g/L of tartaric acid, pH 4.2) was adjusted to pH 3.8 with tartaric acid and exposed to heat treatment (90 °C for 15 min) according to Moreira et al. (2008). The grape must used in this research was steam-sterilised to eliminate its natural microbiota and inactivate its endogenous enzymes in order to study the behaviour of the pectinolytic strain. Subsequently, the must was treated with 80 mg/L sulphur dioxide (as sodium metabisulphite). Concentration of the preservative was chosen because of its lack of inhibitory effect on the yeasts employed in the fermentation trials previously tested (Merín and Morata de Ambrosini, 2015), with the purpose of emulating the winemaking conditions.

2.4. Microvinifications

Microvinifications were carried out in duplicate in 1-L Erlenmeyer flasks containing 800 mL of Malbec red must per replica, in the presence of skins, treated as previously explained (Section 2.3), and conducted in two stages: pre-fermentative cold maceration (PCM; 8 °C–6 days) followed by traditional alcoholic fermentation (TAF; 25 °C–10 days). Three inoculation strategies were applied: (1) pure culture of *A. pullulans* GM-R-22 (control of pectinolytic activity; Ap), (2) pure culture of *S. cerevisiae* IOC 18-2007 (control fermentation; Sc), and (3) mixed culture of both *A. pullulans* GM-R-22 and *S. cerevisiae* IOC 18-2007 (pectinolytic-treatment fermentation; Ap + Sc), with sequential inoculation: the pectinolytic yeast was inoculated at the beginning of PCM and the inoculation of the commercial *S. cerevisiae* strain gave rise to the TAF. Controls were inoculated each with the corresponding microorganism at their respective times of inoculation in the mixed culture. The purpose of the PCM practice in presence of the pectinolytic strain *A. pullulans* GM-R-22 was to enhance the extraction of water-soluble compounds from the solid parts of grape towards the must by means of the cold-active pectinases produced *in situ* by this strain. Monoculture of *A. pullulans* GM-R-22 was not a vinification properly said given that *A. pullulans* species is a non-fermentative microorganism, thus only two wines (Sc wine and Ap + Sc wine) were obtained and analysed.

The flasks were inoculated with the corresponding starter cultures to obtain an initial cell concentration of around 10^5–10^6 CFU/mL for studied yeasts. During the period of skin contact, vinifications were shaken for 20 min at 90 rpm twice a day. Microvinifications were conducted at a controlled temperature using cold chambers (8 °C) or stoves (25 °C).

2.4.1. Progress of the alcoholic fermentation

Vinification performance was monitored by daily measurements of temperature and weight loss of the flasks containing stoppers with a Müller valve that allows only CO_2 to escape from the system up to reach constant weight for two consecutive days. Fermentative power (% alcohol, v/v) was indirectly estimated by multiplying the CO_2 weight loss in grams by a stoichiometric factor of 1.3 (Ciani and Rosini, 1987).

At the end of the fermentations, wines were drained and a cold stabilisation (4 °C) was carried out for 10 days. Then, only free run juice was collected, transferred to glass bottles and kept at 12 °C until analysis.