International Journal of Food Microbiology 190 (2014) 44–53

Contents lists available at ScienceDirect

International Journal of Food Microbiology

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

Microbial communities in air and wine of a winery at two consecutive vintages

Fátima Pérez-Martín [a], Susana Seseña [a,*], Mónica Fernández-González [b], María Arévalo [b], María Llanos Palop [a]

[a] Department of Analytical Chemistry and Food Technology, Environmental Sciences and Biochemistry Faculty, University of Castilla-La Mancha, Avda. Carlos III, s/n, 45071 Toledo, Spain
[b] Department of Analytical Chemistry and Food Technology, Sciences and Chemical Technologies Faculty, University of Castilla-La Mancha, Avda. Camilo José Cela, 10, 13071 Ciudad Real, Spain

ARTICLE INFO

Article history:
Received 12 May 2014
Received in revised form 4 August 2014
Accepted 13 August 2014
Available online 20 August 2014

Keywords:
Lactic acid bacteria
Yeast
Air
Wine
Winemaking process

ABSTRACT

The aim of this study was to assess, both quantitatively and qualitatively, the populations of lactic acid bacteria (LAB) and yeasts in air and wine of a winery, in order to evaluate the possible exchange of microorganisms between them. Samples were taken in a winery located in Castilla-La Mancha (Spain) during the winemaking period of two consecutive vintages (2011 and 2012). The microbial composition was determined by using both a culture-dependent method and a culture-independent method, PCR-denaturing gradient gel electrophoresis (PCR-DGGE). In addition, genetic characterization of isolates from plates was carried out. A high diversity of species was detected in air and wine samples from both vintages. *Leuconostoc mesenteroides* was the predominant lactic acid bacteria in air from both vintages while *Oenococcus oeni* was the predominant in wine. *Saccharomyces cerevisiae* was the most frequently isolated yeast in both air and wine. Typing of *O. oeni* and *S. cerevisiae* isolates from air and wine samples showed the presence of coincident genotypes in both samples, that would confirm the exchange of microorganisms between the two environments, air and wine, and furthermore some of these genotypes were also found at samples taken at different vintages, indicating that they would remain in the winery. The results display the influence of the activity taking place in the winery and the moment of fermentation of the wines in tanks, on the microorganisms present in the air and the role of the air for the dispersal of microorganisms within the winery.

1. Introduction

Winemaking is a complex process based on microbiological transformations including two fermentative stages, the alcoholic fermentation (AF) led by yeast and the malolactic fermentation (MLF) carried out by lactic acid bacteria (LAB). The most accepted sources of both types of microorganisms are grapes and the material in the winery environment (Fleet and Heard, 1993; Mortimer and Polsinelli, 1999). However, some studies (Connell et al., 2002; Garijo et al., 2008, 2009; Ocón et al., 2013a, 2013b, 2013c) have displayed the presence of these microorganisms in the air of the wineries which suggests that it could be a source of both useful and potential contaminant microorganisms, and also the medium for their dissemination. The air acts as a support medium or carrier of microorganisms which travel through it adhered to dust particles, to droplets or as single particles, until they fall and are deposited on the surrounding surfaces (Curiel et al., 2000). Environmental factors such as temperature, dust particle size, humidity and air speed and others such as the area, the activity or the winery design could affect the microbiological composition of the air (Ocón et al., 2013a, 2013b).

There is little information about the composition of the microbiota present in the air of wineries and the few studies carried out have focused on the detection of yeasts, both beneficial and spoilage ones (Connell et al., 2002; Garijo et al., 2008; Ocón et al., 2013a, 2013b, 2013c), and molds (Garijo et al., 2008; Mandl et al., 2008, 2010; Ocón et al., 2011; Simeray et al., 2001). Only Garijo et al. (2008, 2009) studied the presence of LAB in the air, differentiating between *Oenococcus* and non-*Oenococcus* isolates.

In all these reports, with the exception of that carried out by Connell et al. (2002) who used a culture-independent method based in chemiluminescence for detecting *Brettanomyces*, airborne microbial populations have been studied by using culture-dependent techniques, which are well known to offer an incomplete representation of the true bacterial diversity present in an ecosystem since only a small proportion of microorganisms are cultivable (Hugenholtz et al., 1998).

Therefore, it would be interesting to carry out an in-depth microbiological study of the air of a winery by using both culture-dependent and independent methods in order to get a more detailed view of the microbiota present in this ecosystem, which in turn could be considered as representative of the microorganisms present on material surfaces, equipment and stored wines. Knowledge about the quantitative and qualitative diversities of microbial ecology all around the winery and the factors which affect their distribution could offer interesting

* Corresponding author. Tel.: +34 925 265 716; fax: +34 925 268 840.
E-mail address: susana.sprieto@uclm.es (S. Seseña).

http://dx.doi.org/10.1016/j.ijfoodmicro.2014.08.020
0168-1605/

information for the winemakers to develop strategies for the control of spoilage microorganisms in the wineries.

The aim of the present work is to perform a quantitative and qualitative study of lactic acid bacteria and yeasts in the air of a winery and the wines of the nearby fermentation tanks in order to evaluate the possible exchange of microorganisms between them. The study has been carried out in a winery located in Castilla-La Mancha (Spain) during the winemaking period of two consecutive vintages (2011 and 2012). For that, the growth on agar plates using selective media for both types of microorganisms and a culture-independent method, such as PCR-denaturing gradient gel electrophoresis (PCR-DGGE), has been used.

2. Materials and methods

2.1. Sampled area and sample collection

Sampling was conducted in a winery located in Castilla-La Mancha (Spain) which produces and commercializes wine under the Appellation of Origin Méntrida. This winery was selected because it is one of the most important at this area and because it has never used starter cultures for malolactic fermentation.

The winery covers a surface area of 24,000 m^2 presenting two separated areas (Fig. 1): an outdoor area covered by a roof (zone 1) where the vats empty the harvested grapes in a processing facility (barn) and after that they are stemmed, crushed and pumped via pipes; and the vinification area (zones 2 and 3) located inside a two floor building only partially exposed to the outdoor atmosphere through open windows. In it, a number of cement fermenters open at the top (at the level of the second floor) with 1350 hL capacity are arranged as shown in Fig. 1.

Samples of air and wine were taken at two consecutive vintages, from September to November 2011 (22 and 29 September, 19 October and 7 November) and from September to October 2012 (11 and 24 September, 16 and 23 October), between 10.00 am and 13.00 pm. In addition, an air sample was taken between the two vintages (24 May 2012) when there was no activity in the winery.

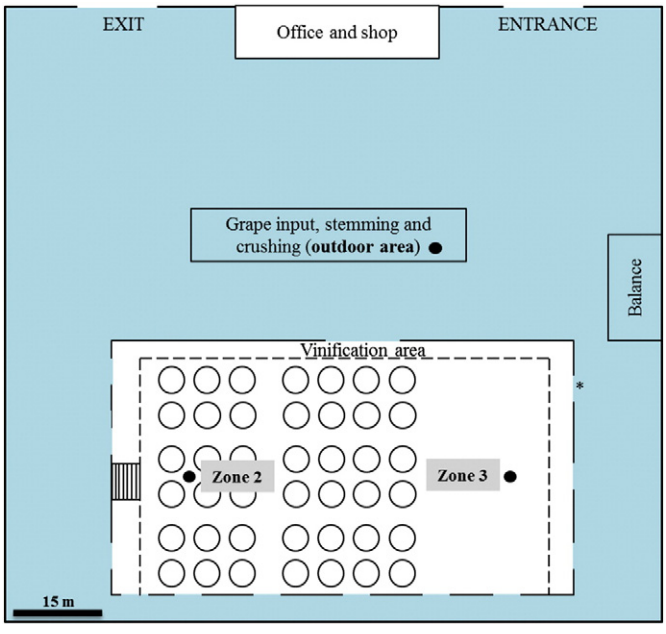

Fig. 1. Diagram of the winery. The color blue indicates outdoor areas. Black circles correspond to the points where the air sampler was placed. *Windows or doors.

Air samples were collected using the AirPort MD8 air sampler (Sartorius Stedim Biotech) that operates according to the filtration and impaction methods. A defined air volume passes through a gelatin membrane filter (Sartorius Stedim Biotech) where the airborne microorganisms are retained. Then, filters can be placed directly on a suitable culture medium in a Petri dish to carry out the counts or dissolved in 10 mL of sterile saline solution (NaCl 0.9%) warmed to 35–40 °C for PCR-DGGE analysis. The volume of air sampled was 1000 L for LAB counts and PCR-DGGE analysis and 250 L for yeast counts. Air samples for both LAB and yeast counts were collected in duplicate.

During both vintages, air samples from three areas were collected: one in the outdoor area (zone 1 in Fig. 1) and two in the vinification area, one of them near full tanks undergoing alcoholic or malolactic fermentations (zone 2 in Fig. 1) and the other one far from such zone (zone 3 in Fig. 1). At the last days sampled at each vintage (19 October 2011, 7 November 2011, 16 and 23 October 2012) only samples in the vinification area (zones 2 and 3) were taken, since the grape input had finished. The air sample between the two vintages was taken at a place between zones 2 and 3.

The air sampler was placed at a distance of approximately 2 m from the crushing unit in the outdoor area and on a platform 1 m above the ground in the vinification area.

At each of these three areas, five samples were taken, two for LAB counts, two for yeast counts and one for PCR-DGGE analysis. In consequence a total of 105 air samples were analyzed.

Wine samples were collected in sterile bottles from the same three fermentation tanks nearest to the zone 2. Counts in duplicate of LAB and yeasts and PCR-DGGE analysis were also performed. A total of 24 samples were analyzed (3 samples × 4 date sampled = 12 samples / vintage). Sampling was coincident with the different moments of the vinification process: before and during AF (22 and 29 September 2011; 11 and 24 September 2012), start and middle of MLF (19 October 2011 and 16 October 2012), end of MLF and emptying of tanks once fermentation was completed (7 November 2011, 23 October 2012).

2.2. Microbial counts

For LAB analysis at air samples, the gelatin membrane filters were placed on MRS agar (Man, Rogosa and Sharpe) (Scharlau, Barcelona, Spain) plates supplemented with 50 mg/L sodium azide and 100 mg/L cycloheximide (Sigma, St. Louis, USA) to prevent growth of acetic acid bacteria and yeast, respectively. The plates were incubated at 30 °C for 5 days under anaerobic conditions (Gas Pack System, Oxoid Ltd., Basingstoke, UK). Cloramphenicol Glucose Agar (CGA) (Scharlau, Barcelona, Spain) plates were used for yeast counts, with plates being incubated at 25 °C for 48 h. In both cases, some diphenyl crystals (approximately 100 mg per plate) (Sigma, Madrid, Spain) were added to the cover plates in order to avoid the development of molds.

After incubation, colonies were counted and the results expressed as the average of the number obtained in the two repeat samples (CFU). Subsequently, this value was converted into the most probable number of microorganisms collected per plate (MPN) by using Feller's law (Feller and Higgins, 1968), following indications of the equipment's instruction manual. Finally, the most probable number of microorganisms collected was expressed as MPN/m^3.

Wine samples were diluted in sterile saline solution and plated in duplicate on MRS agar plates and CGA, as described above. Counts were expressed as colony forming units (CFU) per mL of wine.

A representative number (10%) of isolated colonies from MRS and CGA countable plates from air and wine samples were randomly picked and propagated until purification on MRS agar and YPD (Yeast Extract Peptone Dextrose) (Pronadisa, Laboratorios Conda, Madrid, Spain) plates, respectively. Pure cultures were stored at −80 °C with 20% (v/v) glycerol (Panreac, Barcelona, Spain).