ANNALS OF THE UNIVERSITY OF CRAIOVA

Series: ✓*Biology*
✓*Horticulture*
✓*Food products processing technology*
✓*Environmental engineering*

Vol. XXXI (LXVII) - 2026

RESEARCH ON THE PRESENCE OF YEAST ON THE SURFACE OF GRAPES GROWN IN THE WINE-GROWING REGION BANU MĂRĂCINE

Gagiu (Petroiu) Mihaela[1], Tuțulescu Felicia[2*]
[1]Doctoral School of Plant and Animal Resources Engineering, Faculty of Horticulture, University of Craiova, A.I. Cuza Street, no. 13, 200585 Craiova, Romania, gagiu.mihaela.b3n@student.ucv.ro
[2*]Department of Horticulture and Food Science, Faculty of Horticulture, University of Craiova, A.I. Cuza Street, no.13, 200585 Craiova, Romania
** Correspondence author. E-mail*: felicia.tutulescu@edu.ucv.ro

Keywords*: microorganisms, grapevine, microbial terroir*

ABSTRACT

The grape microbiome is an important factor influencing wine aroma. The primary aromas found in grapes are characteristic of the variety; secondary aromas result from fermentation, while tertiary aromas result from wine aging. Therefore, the microbial and viticultural terroirs are closely linked and very important for the resulting wine. The yeasts present on grapes differ considerably from those that develop in must at various stages of alcoholic fermentation. The isolation and identification of yeasts from a wine-growing region is important because they can serve as the basis for selecting the most valuable strains, adapted to that specific region. This study investigates the presence of yeasts on the surface of grapes located at different heights on the vine as well as in different areas of the grape berry for two varieties cultivated in the Banu Mărăcine wine-growing region.

INTRODUCTION

The microbiota of grapes depends on several factors, such as climate, grape variety, harvest, vineyard characteristics, soil, and viticultural practices (Loureiro, V., et al., 2012; Liu, D., et al., 2019). The diversity of wild yeast and lactic acid bacteria strains is reflected in wine quality (Andorrà et al., 2019). Granchi et al. (2019), studying multiple *Saccharomyces* strains present in consecutive years in a winery, proposed the concept of a "winery effect" or microbial terroir at the sub-regional scale. The use of indigenous yeasts in winemaking contributes significantly to a region's distinct character (Vázquez et al., 2023). The ripeness of the grapes influences the yeast population, which can range from 10^2 to 10^5 CFU per berry (Renouf et al., 2005). New trends in winemaking have emphasized microbiological terroir and led to the use of selected starters from different genera, including non-*Saccharomyces* yeasts or multi-strain starters, to add complexity to the biochemical composition of wines (Mas et al., 2016; 2022). The sequential participation of non-Saccharomyces yeasts in the spontaneous fermentation of wines can benefit the wine, provided that none of these species belong to the category of spoilage yeasts

(Jolly et al., 2014). To impart a specific regional character to wines, an important factor is the exploitation of variations found in the grape microbiota (Lappa et al., 2020). Spontaneous fermentation of grape must can help produce secondary aromas specific to the wine-growing region and the yeasts involved, but it carries the risk of a very slow pre-fermentation phase or even a complete blockage of this phase, because the non-*Saccharomyces* yeasts that predominate on the grapes initiate alcoholic fermentation but cannot produce more than 3–4% alcohol by volume. Fermentation arrest occurs when Saccharomyces yeasts—which are present in small numbers on the grapes—struggle to multiply in the must and consequently delay the onset of fermentation. A decrease in the number of yeast cells on the surface of the grapes may be a consequence of fungicide application during the growing season (Comitini et al., 2008). Global warming has led to harvests with higher sugar concentrations and lower acidity, which can slow down alcoholic fermentation (Sun, Q., et al., 2023). Determining the optimal time to harvest the grapes is the winemaker's decision. This varies depending on the type of wine they wish to produce. Thus, one can speak of "phenolic ripeness" when the harvest is intended for red wines, and "aromatic ripeness" when the grapes are intended for aromatic wines. The sugar content accumulated in the grapes and their acidity are also decisive factors in determining the optimal time to harvest the grapes (Fugelsang et al., 2007; Meléndez et al., 2013).

MATERIAL AND METHODS

The research was conducted in the Banu Mărăcine wine-growing region, which is part of the Dealurile Craiovei vineyard (44°19′ north latitude and 23°48′ east longitude, at an elevation ranging from 176 to 190 meters above sea level). The grape varieties studied were *Chardonnay* and Merlot. Both varieties were planted in 2016 and have been bearing fruit since 2020, indicating that a specific microbiota has developed. This study presents the results obtained from the microbiological analysis of grapes harvested before full maturity, specifically approximately 20 days before the start of the harvest. For the microbiological analysis, two bunches were harvested from each of 10 vines selected to cover the entire cultivated area, including the edge of the vineyard, its center, the top of the slope, and the base of the slope. The exposure of the vine row affects the ripening of the grapes as well as sugar accumulation. The sugar content of the harvested grapes was determined using the refractometric method. This is a physical method of analysis based on the principle of light refraction at 20°C. Two clusters were harvested from each vine—one from the base of the vine and one from the top—since it is known that yeasts reside in the soil, from where they spread to the grapes as they ripen. Yeast isolation was performed by swabbing two areas of the grape berry using a sanitizing swab: the area near the peduncle and the area at the tip of the berry. The grapes were harvested into sterile bags and immediately transported to the laboratory, where they underwent microbiological analysis on the same day. Inoculation was performed on YPGA medium (1 % yeast extract, 2 % peptone, 2 % glucose, 2 % agar, MERK) incubated at 27 °C for 48 hours. Typical colonies were subsequently transferred to another Petri dish containing the same culture medium; after several transfers, pure cultures were obtained and subjected to standard identification tests (morphological, physiological, and biochemical characteristics). Based on these characteristics, the colonial isolates were classified as either sporogenic or non-

sporogenic yeasts. This characteristic is very important, given that sporogenic yeasts exhibit very intense fermentative activity, compared to non-sporogenic yeasts, which can produce a maximum of 5% vol. of alcohol. Moulds isolated from grapes were identified using cultural methods (colony appearance) and morphological methods (microscopic appearance).

RESULTS AND DISCUSSIONS

The sugar concentration of the grapes was calculated as the average for the clusters harvested from the base and the top of the vine, respectively. Table 1 shows the sugar concentration of the grapes harvested 20 days before full maturity. The data in Table 1 show that grapes at the top of the vine accumulate more sugar than those at the base of the vine. This can be explained by exposure to sunlight and the intensity of photosynthesis.

Table 1. Sugar concentration in grapes (%)

Variety	The sugar concentration in the grapes positioned at the bottom of the vine trunk (%)	The sugar concentration in the grapes positioned at the top of the vine trunk (%)
Chardonay	21	24
Merlot	22	25

With regard to the number of microorganisms, expressed as CFU/cm² of grape berry surface area, it was found that the number of microorganisms is higher in the peduncle region than at the tip of the grape berry for both varieties studied (Figs. 1–2).

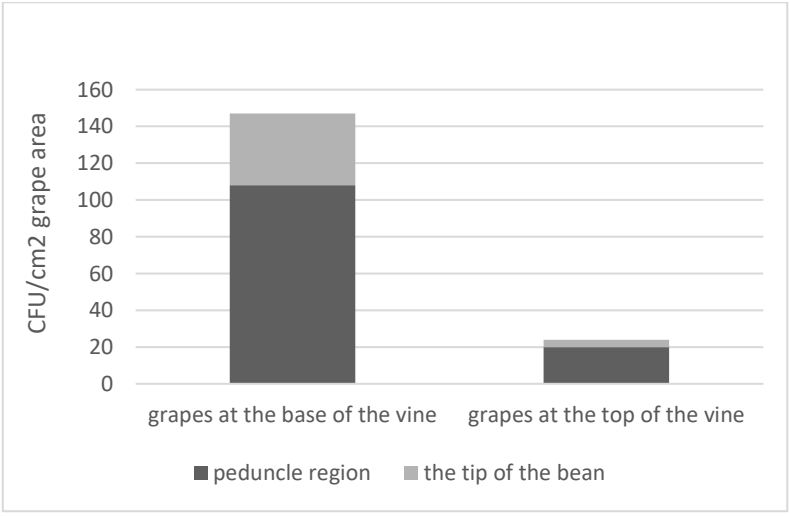


Fig. 1. Yeast count (CFU/cm²) on the surface of a grape berry (*Chardonnay* variety)

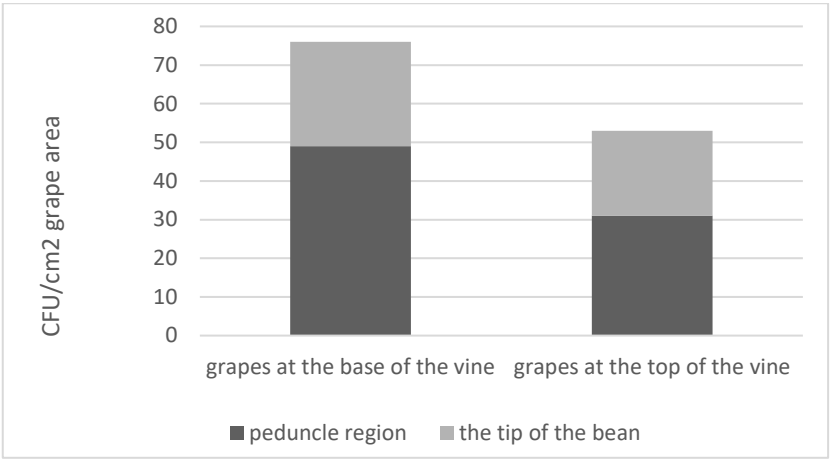


Fig. 2. Yeast count (CFU/cm²) on the surface of a grape berry (*Merlot* variety)

It has been observed that although the grapes at the top of the vine accumulate more sugars, the higher concentration of yeast is found on the grapes located at the base of the vine, which demonstrates that the yeast migrates from the soil. The closer the grapes are to the soil, the higher the yeast load. In the *Chardonnay* variety, a greater difference is observed in the microbiota of the grapes at the base of the vine compared to those at the top, compared to the *Merlot* variety. Figures 1–4 show macroscopic aspects of the colonial isolates for the two varieties studied.

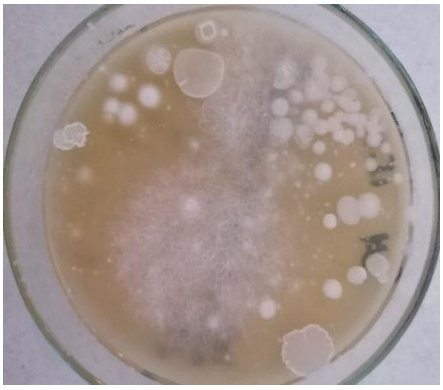


Photo 1. Colonial isolates from grapes *Chardonnay* variety – clusters located at the base of the vine



Photo 2. Colonial isolates from grapes *Chardonnay* variety – clusters located at the top of the vine



Photo 3. Colonial isolates from grapes *Merlot* variety – clusters located at the base of the vine

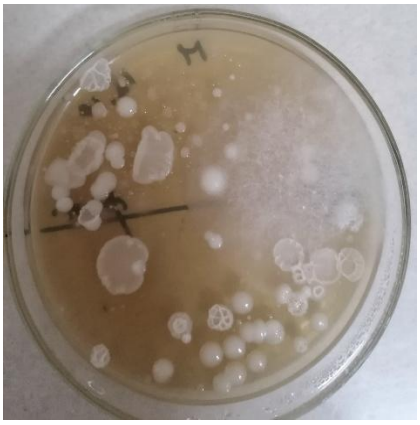


Photo 4. Colonial isolates from grapes *Merlot* variety – clusters located at the top of the vine

It is worth noting that, although the harvested grapes showed no visible fungal infections, mold spores present on the surface of the grapes did form colonies on the culture medium. These spores belonged to the genus *Alternaria* (Photo 5).

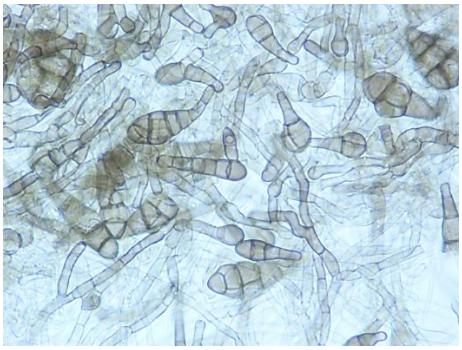


Photo 5. *Alternaria spp*. isolates from grapes

The predominant microbiota of Chardonnay grapes consists of genera belonging to the non-Saccharomyces group, specifically non-sporulating yeasts (Fig. 3). These are predominant on grapes located at the base of the vine and in the peduncle area. Sporulating yeasts, on the other hand, are found in greater proportions at the tip of the grape berry. This can be explained by the easier spread of spores and their resistance to conditions that are unfavorable to non-sporogenic yeasts. Regarding the relative abundance of yeasts present on the grapes at the base and the top of the vine, it can be observed that the proportion of sporogenic yeasts increases in the grapes located at the top of the vine, compared to those at the base.

In the Merlot variety cultivated in the Banu Maracine wine-growing region, as is the case with the Chardonnay variety, non-sporogenic yeasts predominate over sporogenic ones, especially on the grapes at the base of the vine. With regard to the

area of the grape berry from which the isolation was performed, non-sporogenic yeasts predominate over sporogenic ones, especially in the peduncle region (Figs. 4–5).

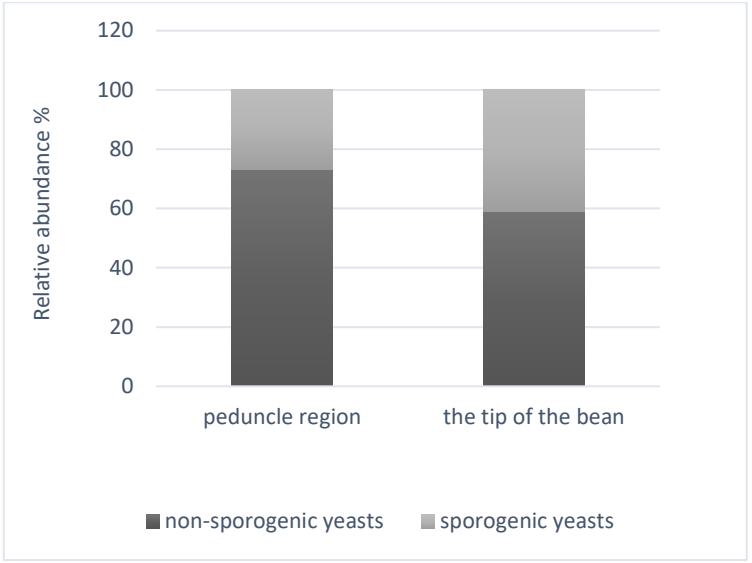


Fig. 3. Relative abundance of sporogenic and non-sporogenic yeasts on grapes at the base of the vine (*Chardonnay* variety)

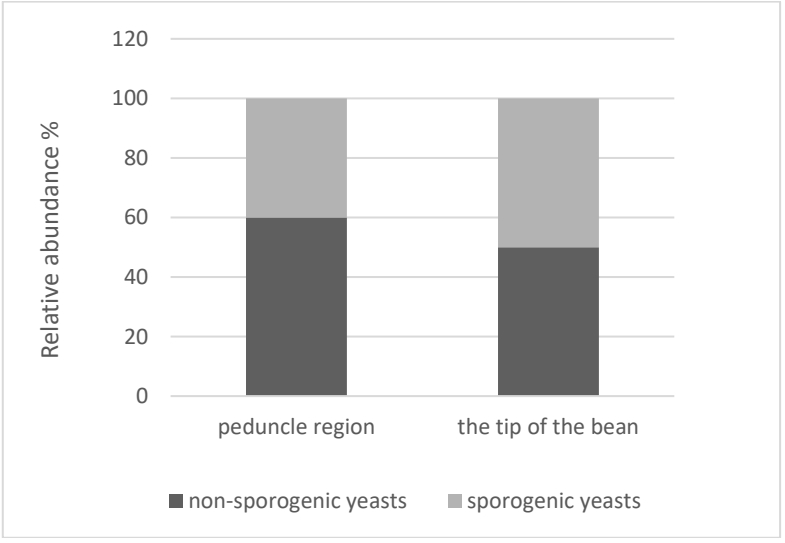


Fig. 4. Relative abundance of sporogenic and non-sporogenic yeasts on grapes from the upper part of the vine (*Chardonnay* variety)

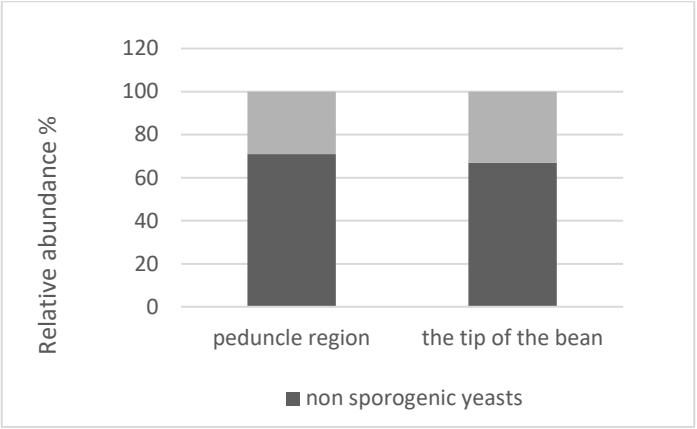


Fig. 5. Relative abundance of sporogenic and non-sporogenic yeasts on grapes at the base of the vine (*Merlot* variety)

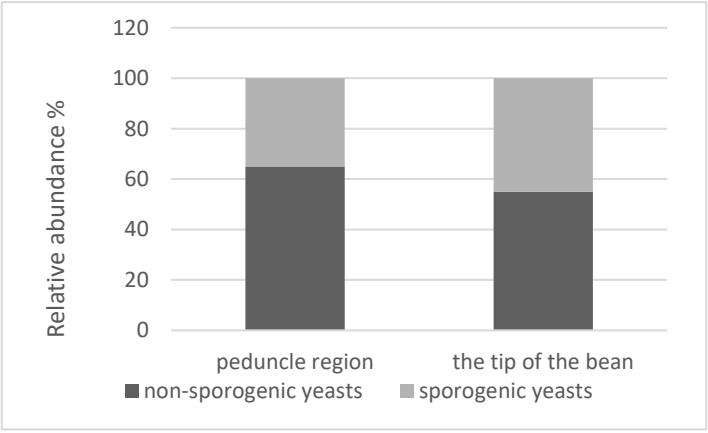


Fig. 6. Relative abundance of sporogenic and non-sporogenic yeasts on grapes from the upper part of the vine (*Merlot* variety)

It has been observed that sporogenic yeasts are found in greater numbers on the grapes at the top of the vine than on those at the base. At this stage of grape ripening, non-sporogenic yeasts are more numerous than sporogenic ones.

CONCLUSIONS

This study highlights the presence of yeasts on the surface of grapes, as well as their relative abundance depending on the anatomical region of the berry. The results demonstrate that the grape microbiota can be influenced both by the position of the cluster on the vine and by the anatomical region of the grape berry. The closer the clusters are to the base of the vine, the larger the yeast population and the greater the proportion of non-sporogenic genera. Sporogenic yeasts are more abundant on clusters located at the top of the vine. The anatomical region of the grape berry influences the number of microorganisms and whether they belong

to the sporogenic or non-sporogenic group. The peduncle region is richer in yeasts compared to the tip of the grape berry.

REFERENCES

Andorrà, I., Miró, G., Espligares, N., Maria Mislata, A., Puxeu, M., Ferrer-Gallego, R. 2019. Wild yeast and lactic acid bacteria of wine. In yeasts in biotechnology; Peixoto Basso, T., Ed.; IntechOpen: London, UK.

Comitini, F., Ciani M. 2008. Influence of fungicide treatments on the occurrence of yeast flora associated with wine grapes. Annals of Microbiology, 58 (3) 489-493

Fugelsang, K.C., Edwards, C.G.2007. Wine microbiology: practical applications and procedures, 2nd ed.; Springer: New York, NY, USA.

Granchi, L., Ganucci, D., Buscioni, G., Mangani, S., Guerrini, S. 2019. The biodiversity of Saccharomyces cerevisiae in spontaneous wine fermentation: The occurrence and persistence of winery-strains. Fermentation 5, 86.

Jolly, N.P., Varela, C., Pretorius, I.S. 2014. Not your ordinary yeast: non-*Saccharomyces* yeasts in wine production uncovered. FEMS Yeast Res. 14, 215–237.

Lappa, I.K., Kachrimanidou, V., Pateraki, C., Koulougliotis, D., Eriotou, E., Kopsahelis, N. 2020. Indigenous yeasts: emerging trends and challenges in winemaking. Curr. Opin. Food Sci. 32, 133–143.

Liu, D., Zhang, P., Chen, D., Howell, K. 2019. From the vineyard to the winery: how microbial ecology drives regional distinctiveness of wine. Front. Microbiol. 10, 2679.

Loureiro, V., Ferreira, M.M., Monteiro, S., Ferreira, R.B. 2012. The microbial community of grape berry. In The biochemistry of the grape berry. Eds.; Bentham Science: Oak Park, IL; pp. 241–268.

Mas, A., Guillamón, J.M., Beltran, G. 2016. Non-conventional yeast in the wine industry. Front. Microbiol. 7, 1494.

Mas, A., Portillo, M.C. 2022. Strategies for microbiological control of the alcoholic fermentation in wines by exploiting the microbial terroir complexity: A mini-review. Int. J. Food Microbiol. 367, 109592.

Meléndez, E., Ortiz, M.C., Sarabia, L.A., Íñiguez, M., Puras, P. 2013. Modelling phenolic and technological maturities of grapes by means of the multivariate relation between organoleptic and physicochemical properties. Anal. Chim. Acta 761, 53–61.

Renouf V., Claisse O., Lonvaud-Funel A. 2005. Understanding the microbial ecosystem on the grape berry surface through numeration and identification of yeast and bacteria. Australian Journal of Grape and Wine Research 11, 316–327.

Sun, Q., Granco, G., Groves, L., Voong, J., Van Zyl, S. 2023. Viticultural manipulation and new technologies to address environmental challenges caused by climate change. Climate 11, 83.

Vázquez, J., Mislata, A.M., Vendrell, V., Moro, C., De Lamo, S., Ferrer-Gallego, R., Andorrà, I. 2023. Enological suitability of indigenous yeast strains for 'Verdejo' wine production. Foods. 12, 1888.