CENTENARY OF THE OIV
45th WORLD CONGRESS OF VINE AND WINE
14-18 OCTOBER 2024 FRANCE-DIJON
OIV
Available on IVES Conference Series vine & wine
DOI : https://doi.org/10.58233/S84nQWQ0

Are all red wines equals regarding their vulnerability to *Brettanomyces bruxellensis*?

Julie Miranda[1], Laura Olazabal[1], Eglantine Alves Gonçalves Lopes[1], Benjamin Allaume[1], Cécile Miot-Sertier[1], Caroline Rouger[1,2], Marguerite Dols-Lafargue[1,3]

[1] Univ. Bordeaux, INRAE, Bordeaux INP, Bordeaux Sciences Agro, OENO, UMR1366, ISVV, F-33140 Villenave-d'Ornon, France
[2] Bordeaux Metabolome, MetaboHUB, PHENOME-EMPHASIS, Centre INRAE de Nouvelle Aquitaine-Bordeaux, F-33140, Villenave-d'Ornon, France
[3] ENSMAC, Bordeaux INP, 33600, Pessac, France

Abstract. In recent years, many studies have been conducted to better understand how contamination by the spoilage yeast *Brettanomyces bruxellensis* occurs in wine. This yeast, which drives the formation of volatile phenols, is well adapted to the wine difficult environment: some strains resist to high alcohol, low pH, and sulphites, and are very difficult to eliminate. However, the vulnerability of the wines, ie, their ability to promote *B. bruxellensis* growth, was reported by winemakers to change depending on the vintage or the winery considered. The aim of this study was to quantify and objectively compare the wine vulnerabilities by analysing the growth of 5 strains of *B. bruxellensis* in 69 wines from the Bordeaux region. Then the impact of several factors putatively responsible for the "permissiveness" such as the vintage or the variety was studied.

1. Introduction

Since its discovery in 1992 [1], *Brettanomyces bruxellensis* is recognised as a main problem for winemakers worldwide. This yeast is known to produce volatiles phenols in wine, especially ethyl phenols associated, when present in wine, with unwanted odours such "horse sweat", "leather" or "drug". Their presence in wine can also reduce the fruity aroma of the wine, making this yeast one of the most feared spoilage microorganisms encountered during winemaking. *B. bruxellensis* is particularly well adapted to the wine environment, with low nutrient requirements and a high resistance to stresses prevailing in wine, such as high alcohol and low pH. Furthermore, some strains are highly resistant to sulphite, the most commonly used antimicrobial in cellars. Interestingly, a few winemakers reported that specific wines in specific domains were spoiled more often than others, while other wineries reported a low contamination history. Therefore, in this study, we tried to answer these following questions: do red wines have different abilities to support *B. bruxellensis* development and how can the vulnerability of the wine be defined? Which factors modulate the different "permissivenesses" observed in wine?

2. Materials and methods

2.1. Wines

In this study, 69 red wine samples were collected from the Bordeaux region. In total, six wineries participated and gave 63 monovarietals wines that were sampled immediately after the end of the malolactic fermentation and before barrelling. Six blended wines were also collected between one and two months after the end of the malolactic fermentation. Three varieties were studied: Cabernet Sauvignon, Merlot, and Cabernet franc. Most of these wines belonged to the 2020 and 2021 vintages, but 14 were collected in the 2022 and 2023 vintages.

After reception, the wines were finned with egg white [2] and pasteurised (80°C for 30 min) before microbiological analysis. The absence of any microorganisms present after pasteurisation was checked using the same method as described in section 2.2 strains and growth analysis.

2.2. Strains and culture conditions

To evaluate the behaviour of *B. bruxellensis*, five strains isolated from wine were used: AWRI 1499, CRBO L0424, CRBO L0422, CBS 2499, and CRBO L0611. Two of them belonged to the AWRI 1499 like genetic group of triploid strains, one to the AWRI 1608 like genetic group of triploid strains and the last two, to the CBS 2499 like genetic group of diploid strains. One by one, the strains were gradually adapted to all 69 wines at 25°C before inoculation at 10^2 UFC/ml according to previous work [3].

2.3. Growth conditions

The growth curves for each strain in each wine were obtained after plating and counting the cultivable populations present in the wine over 6 to 20 weeks according to a previously described method [4]. From each curve, three parameters were calculated: lag phase, maximal growth speed, and maximal population, still in accordance with a previous study [4]. Experiments were conducted until three consecutive identical population levels were observed.

2.4. Statistical analysis

All statistical analyses were performed using RStudio (version 1.4.1717; RStudio Team, Boston, MA, USA) with a significance level of 5%. Assessment of the factor's significance was performed using ANOVA, and normality was checked using normality and Levene's test.

3. Results and discussion

To answer to the first question (do red wines have different abilities to support *B. bruxellensis* development), the growth profiles of the 5 strains were analysed in all 69 wines (69 × 5 growth curves examined). Then, a notation system was created to assign a unique value comprised between 0 and 40 to each wine, 40 being a score associated with a highly permissive or vulnerable wine and 0 to a very unfavourable one. Then, using the notation, the influence of multiple factors (vintage, winery, variety, and wine) on wine vulnerability was studied.

3.1. *B. bruxellensis* growth behaviour in wines

The comparison of the growth of the five strains in 69 wines allowed us to establish four growth profiles, that is to say four classes of wines (figure 1).

In the wines categorised under profile 1, the five strains began growing immediately without any noticeable lag phase (growth occurring between 0 and 7 days), and reached their maximum population quickly (in less than 40 days). All strains reached a population of 10^6 UFC/ml. This group of wines, defined as the very permissive ones, included the majority of the wines examined, specifically 37 out of 69 (54%).

Profile 2 included wines in which at least one of the five strains experienced growth difficulties, showing a lag phase before growth. This, often coupled with a slower growth rate for diploid strains, extended the duration of the experiment. However, the maximum population was comparable to that observed with profile 1 wines. This profile encompassed 12 wines (17%).

In profile 3 wines, no growth was observed for the diploid strains, whereas the triploid strains initially exhibited a significant lag phase before growing to a maximum population similar to profiles 1 and 2. Only a few wines, six out of 69 (9%), fell into this category.

Wines with profile 4 supported no growth or the growth of only one triploid strain, with a noticeable drop in the cultivable population below the detection threshold (10^2 UFC/ml), often observed after the first week. A fifth profile was identified, in which four out of the five strains even failed to survive the adaptation process. Because this profile only included four wines out of the 69 studied, profiles 5 and 4 were merged and defined as gathering the 14 less-permissive wines (20%).

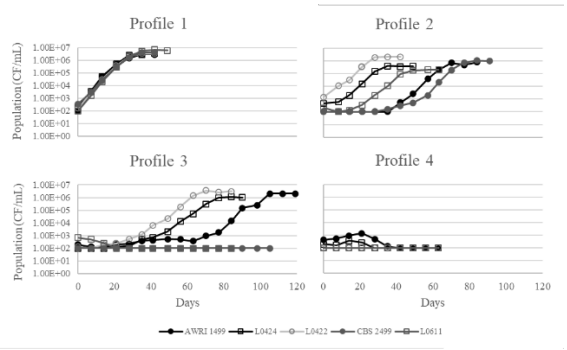

Figure 1. Distinct growth profiles observed. For each profile, the results obtained with one representative wine is shown. In profiles 1 and 4, all the strains display the same behaviour, while they distinguish in wines with profile 2 and 3.

Examining the five growth profiles clearly indicated that the Bordeaux red wines studied vary in their ability to support *B. bruxellensis* growth. This also confirmed that certain *B. bruxellensis* strains, particularly triploid ones, are better adapted to the conditions found in Bordeaux wines, as suggested [5,6] and supported [7–9] by multiple authors. The difference between triploid and diploid strains was expected to be even more pronounced in the presence of added sulphites which was not present in our studied wines. The profile attribution allowed for an initial classification of the wines. Based on the volatile phenol production rates reported in the literature, wines with profile 1 could be spoiled by phenol concentrations exceeding the perception thresholds within 3–4 weeks. For profile 2 wines, this would take 40–75 days, and for profile 3 wines, 75 to over 120 days. For profile 4 wines, this phenomenon did not occur within 120 days. The growth delay could also delay the phenol production and thus give more time to the winemaker to react after having detected the presence of *B. bruxellensis*.

3.2. Evaluation of the wine permissiveness towards *B. bruxellensis*

Each wine could be fitted into one of the four profiles, but to facilitate the study of the growth behaviour, the curve had to be turned into a single value for each wine; hence, the necessity of creating a permissiveness notation to better discriminate the wines.

To do so, three growth parameters were turned into marks (Table 1). The value 0 was attributed to a - an important lag phase, a null growth speed, and to low final population. In contrast, a value of 3 (except for the population parameter), meant that there was no lag phase, a high growth speed, and a final population of over 10^6 UFC/mL.

Table 1. Notation grid. A high note indicates that B. bruxellensis growth is easy and efficient in the considered wine. Four levels are considered for the lag phase and the growth rate and three only for the maximal population.

Note	Lag phase (days)	Growth rate ($days^{-1}$)	Maximal population (CFU/ml)
0	120	0	$<10^4$
1	43 < & < 120	< 0.4	10^4 < & < 10^6
2	0 < & < 43	0.4 < & < 0.6	$>10^6$
3	0	> 0.6	

For each growth curve, these 3 marks were summed to create an intermediate score, called Sum1. The Sum1 score reflects the growth efficiency of one strain in a single wine.

The 5 "Sum1" associated with the growth of the 5 strains in a same wine were then summed to obtain the permissiveness score of this wine. A score close to 0 means the wine is non permissive as most *B. bruxellensis* strains failed to develop. On the contrary, a score close to 40 means that the wine is very vulnerable in case of contamination by the yeast. The details of this notation can be found in article [4].

3.3. Influence of external factors

With the help of the notation system, a statistical analysis was performed to evaluate the impact of factors known to modulate the wine composition: (i) the vintage (that includes the climate variation and all sub variables that depends of this factor), (ii) the grapes variety, and (iii) the winery, that encompasses the winemaking itinerary, but also a "terroir" notion.

With the intermediate score, "Sum1", the influence of the strain could be evaluated. An ANOVA was thus performed on the 69 × 5 "Sum1" obtained (figure 2.A).

Most of the variation observed in the Sum1 was due to the wine (27%), to the winery (26%), and to the vintage (22%). The strain was only responsible for 4% of the explained variation of Sum1. A closer examination of the wine factor (figure 2.B), showed that most of the wine effect (82%) was indeed due to the alcohol level. The mean ethanol content of the wines with a score below 16 was 14.5%. This alcohol effect may also explain part of the strain effect, because strains in the AWRI 1499 like group are known to cope with high alcohol levels better than all the others [6].

However, 18% of the wine factor could not be explained by alcohol or pH, which had no impact in this study. This could mean that another factor plays a role in wine vulnerability. This could be the wine chemical composition, since another factor is the winery. The difference between wineries encompasses the "terroir" and the winemaking process (macerations, microbial starters, filtrations, use of barrels…) that could alter the wine composition [10–13].

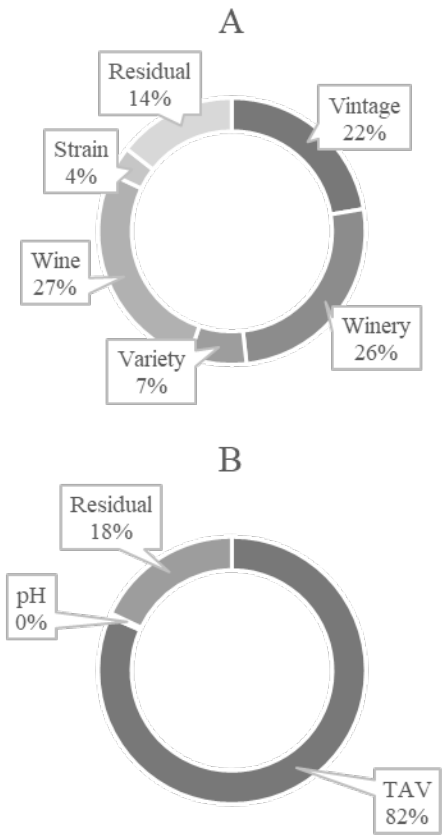

Figure 2. ANOVA to evaluate the impact of each factor on the score.

4. Conclusion

In this study, we demonstrated the existence of a high level of diversity among Bordeaux wines, some wines being more susceptible to *Brettanomyces bruxellensis* than others. These differences in vulnerability can be attributed to factors such as alcohol content and wine composition, which depend on the winemaking process and unique characteristics of the terroir.

Future research should focus on identifying the specific elements of wine composition that contribute to this variability, beyond the influence of ethanol levels. By examining the chemical differences linked to these factors, we aim to better understand and anticipate the risks in case of *B. bruxellensis* contamination in Bordeaux wines. These results could then be expanded to all red wines in the world

5. References

1. . P. Chatonnet, D. Dubourdieu, J.N. Boidron, M. Pons, J Sci Food Agric, 60 (1992)
2. P. Ribéreau-Gayon, Traité d'Oenologie, 1, (2017)
3. A. Cibrario, M. Avramova, M. Dimopoulou, M. Magani, C. Miot-Sertier, A. Mas, et al.. PLoS ONE, 14, (2019)
4. J. Miranda, C. Miot-Sertier, L. Olazabal, W. Albertin, T. Richard, G. Da Costa, et al., Food Control, 155, (2024)
5. A. Cibrario, C. Miot-Sertier, L. Riquier, G. de Revel, I. Masneuf-Pomarède, P. Ballestra, et al., Am. J. Enol. Vitic, 71, (2020)
6. A. Cibrario, C. Miot-Sertier, M. Paulin, B. Bullier, L. Riquier, M.C. Perello, et al., Food Microbiol, 87, (2020)
7. C. Longin, C. Degueurce, F. Julliat, M. Guilloux-Benatier, S. Rousseaux, H. Alexandre, Food Res. Int, 89, (2016)
8. M. Avramova, A. Vallet-Courbin, J. Maupeu, I. Masneuf-Pomarède, W. Albertin. Front Microbiol, 9, (2018)
9. J. Harrouard, C. Eberlein, P. Ballestra, M. Dols-Lafargue, I. Masneuf-Pomarede, C. Miot-Sertier, et al., Mol. Ecol, 32, (2023)
10. M. Dimopoulou, V. Troianou, C. Toumpeki, Y. Gosselin, É Dorignac, Y. Kotseridis, OENO One, 54, (2020)
11. F. Bekris, D. Lola, E. Papadopoulou, S. Vasileiadis, S. Paramithiotis, Y. Kotseridis, et al., LWT, 205, (2024)
12. A. Baiano, C. Terracone, F. Longobardi, A. Ventrella, A. Agostiano, M.A. Del Nobile, Food Chem, 135, (2012)
13. 1 F. Orbanić, S. Rossi, E. Bestulić, I. Budić-Leto, K. Kovačević Ganić, I. Horvat, et al., Foods, 12, (2023)