INTERLABORATORY TESTS TO ASSESS MICROBIOLOGICAL QUALITY OF WINE AS A MEANS TO PREPARE FOR THE INFLUENCE OF CLIMATE CHANGE ON THE WINEMAKING PROCESS

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INTRODUCTION

Climate change will likely lead to the appearance of new microbial species during the winemaking process, and laboratories have therefore a growing need to control these phenomena.

These laboratories are expected to perform quantitative and qualitive microbiological analyses on wine with precision, so interlaboratory tests are an effective and important means of ensuring the validity of results.

BIPEA, as a proficiency testing provider, offers laboratories the opportunity to evaluate themselves and improve their ability to use the methods designed for the study of wine microbiology, to prevent alterations, and to monitor the winemaking process.

In the PTS (Proficiency Testing Scheme) dedicated to these analyses, launched in 2016, the number of participants has grown in tandem with laboratory interest in this topic. The list of microorganisms tested and the methods applied have also expanded over time to meet the needs of professionals, thanks to feedback from participants.

Several microbial groups, including *Brettanomyces* and lactic acid bacteria (among others), are tested using Pasteurian culture methods and genomic methods such as PCR.

METHODOLOGY

The implementation of a proficiency test can be schematized by 3 main steps: preparation of samples, analysis by laboratories, and statistical treatment of the data.

SAMPLE PRODUCTION

One of the fundamental requirements for the implementation of a PT is the preparation of homogeneous and stable samples. For this PT, four series of samples were prepared by respectively spiking a batch of homogenized wine with a suspension of *Dekkera bruxellensis, Acetobacter aceti, Lactobacillus fermentans,* and another one with both *Aspergillus brasiliensis* and *Saccharomyces cerevisiae* in well-controlled proportions.
According to the requirements of the ISO 13528, [1] homogeneity of the samples was verified by experimental studies on 10 samples *in duplo* taken randomly across a batch of samples. Stability of the product was demonstrated by analyzing 3 samples *in duplo* for 7 days. For both studies, the analyses were performed according to the Compendium of the OIV (International Organisation of Vine and Wine [2]).

ANALYSIS

Samples were shipped at 5±3 °C to the laboratories participating in the test together with an indicator for monitoring the temperature.
A reply form was made available to allow the laboratories to return their analysis results.
Moreover, participants were invited to enter in the reply form some complementary information, such as the date of the beginning of the analysis, the method used, the growth medium used, the incubation temperature, and the time and type of plating.
Given the inherent instability of microbiological samples, the participants were encouraged to analyze the samples as soon as possible after reception.

STATISTICAL TREATMENT

The statistical treatments were conducted according to the ISO 13528 standard [1]. The assigned values (x_{pt}) were estimated using the robust means of the results. The proficiency of each laboratory was evaluated thanks to tolerance values (TV) of twice the standard deviation for each parameter. Each result (x) could then be evaluated through z-scores:

z ≤ |2| : satisfactory
|2| < z ≤ |3| : questionable
z > |3| : unsatisfactory

Where : $z=(x-xpt)/(\frac{TV}{2})$

RESULTS and DISCUSSION

Since there is evidence of evolution in both the quantity and the variety of microorganisms in wine concurrently with climate evolution ([3] [4]), there is a pressing need for laboratories to practice on wider lists of microorganisms and at different concentration levels. In order to follow the evolution of their needs, meetings are organized regularly to discuss and collect the requests of laboratories. The design of the tests can then be adapted to meet their needs and remain relevant for the profession.
At first, only *Brettanomyces* and lactic bacteria were present in the samples. Additional microorganisms were added at the end of 2017 (acetic bacteria) and 2020 (yeast and mold).
Laboratory interest has grown over the years and participation in the tests has increased steadily (Fig. 1).

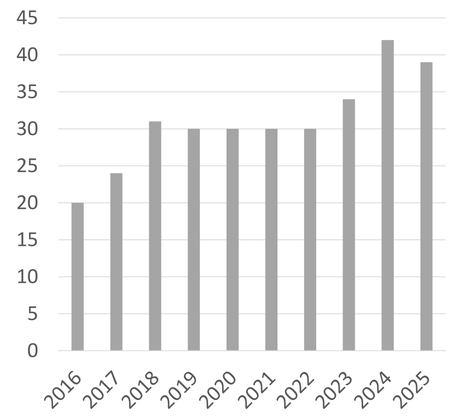

Fig. 1: Participation over time in the tests

An analysis of the results obtained from May 2023 to February 2025 is shown graphically in Fig 2 & 3. The participants with less than or equal to 20% of unsatisfactory results over the last six tests represent 54% of the population if we consider all possible parameters for this PT, indicating that there is room for improvement. There are large disparities in both the performance and the number of results provided by laboratories when all parameters are considered (Fig. 2). In particular, the laboratories that gave fewer results seemed to have more difficulties during the tests in the period studied, as 67% of the laboratories that gave more than 20 results had less than or equal to 20% unsatisfactory results from the total provided.
Saccharomyces seems to be the least popular analysis, while the other parameters receive a comparable number of responses on average with the Pasteurian growth method (PCM). It should be noted that the PCR method received far fewer responses than the Pasteurian growth method when both were available (Fig. 3). It was possible to calculate an assigned value for most of the parameters for these tests, with the exception of *Saccharomyces* with both methods (all tests) and lactic bacteria with the PCR method (three out of six tests) leading to the absence of evaluation due to an insufficient amount of data. No parameter reached more than 20% untrue results, which is satisfactory.

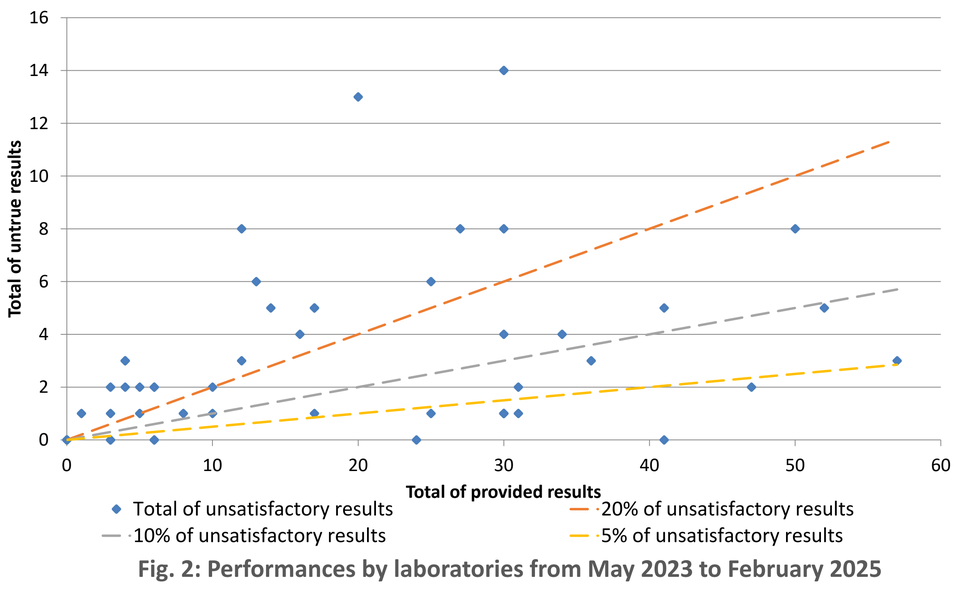

Fig. 2: Performances by laboratories from May 2023 to February 2025

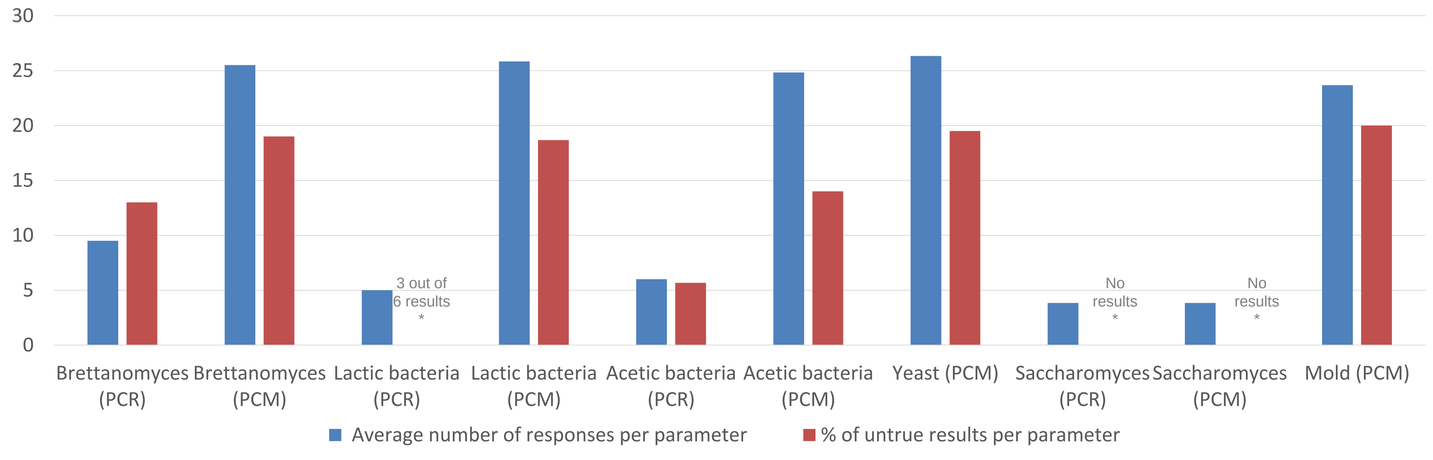

Fig. 3: Participation and performances from May 2023 to February 2025

CONCLUSION

Overall, the performance per parameter remains satisfactory, though disparities between laboratories have appeared in recent years, suggesting that the methods used are better mastered by some laboratories than others. The addition of new species or strains to the samples allows laboratories to better prepare for upcoming challenges with the increasing risk of detrimental microbiological activity in wine.
Laboratory interest seems greater for the Pasteurian growth method compared to PCR. This may originate from the difference of cost between the two, where qPCR is faster but also more expensive. For the moment, few laboratories analyze *Saccaromyces,* but higher temperatures may induce a shift in yeast population dynamics and consequently a need for monitoring. [5]

REFERENCES

[1]: ISO 13528:2015 - Statistical methods for use in proficiency testing by interlaboratory comparisons
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[3] Ramón Mira de Orduña, Climate change associated effects on grape and wine quality and production, Food Research International, Volume 43, Issue 7,2010,Pages 1844-1855,ISSN 0963-9969.
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