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Does *Saccharomyces cerevisiae* play a supporting role in mousy off-flavours production?

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The alteration known as "mousy taint" is a resurgent deviation in certain wines, characterised by reminiscent aromas of rice cakes and sausage skins. *Saccharomyces cerevisiae*, the microorganism responsible for alcoholic fermentation in wines, is crucial for winemaking and has not been directly linked to this defect. However, recent studies suggest that some strains of *S. cerevisiae* may indirectly affect the production of mousy *N*-heterocycles in a synthetic medium.

Introduction

The "mousiness" defect is marked by aromas that range from "basmati rice" to "mouse urine" or "sausage skin". It is only detectable retronasally due to the low volatility of the three N-heterocycles responsible for the alteration (2-acetyltetrahydropyridine, ATHP; 2-ethyltetrahydropyridine, ETHP and 2-acetylpyrroline, APY)[1]. These compounds can be produced by lactic acid bacteria, such as *Lentilactobacillus hilgardii* and *Oenococcus oeni*, as well as the yeast Brettanomyces bruxellensis, which is also known for producing volatile phenols in wines[2] [3]. Although Saccharomyces cerevisiae cannot produce the compounds responsible for "mousy taint", it is worth noting that after alcoholic fermentation the yeast persists throughout the rest of the winemaking process and in the final product. To date, the production of mousiness by microorganisms has only been tested in pure culture, and not on a consortium of microorganisms. Therefore, Moulis *et al.* (2024)[4] centred their work on the contribution of consortia of microorganisms to mousiness. This paper focused on three B. bruxellensis strains in the presence or absence of three S. cerevisiae strains in a N-heterocycle assay medium (*N*HAM) (Table 1).

Results

The study by Moulis *et al.*, 2024[4], investigated the potential impact of S. cerevisiae on the mousy aroma compounds produced by B. bruxellensis. Chemical analyses of mousy N-heterocycles were performed by SBSE-GC-MS. Due to a technical issue, the calibration line associated with these analyses is not available, and only qualitative results that compare the peak areas of the molecule relative to the internal standard (IBMP-d3) are presented here.

When screened individually, the three strains of *S. cerevisiae* tested did not produce any of the three *N*-heterocycles in the *N*HAM (Figure 1).

According to the literature, *B. bruxellensis* does not produce APY[2,5], even in the presence of *S. cerevisiae*. Conversely, all three strains of *B. bruxellensis* (YJS 7816 from the Laboratory for Molecular Genetics, Genomics and Microbiology, Strasbourg University, France, and CRBO L0417 and CRBO L1751 from the Centre de Ressources Biologiques Œnologique, ISVV, Bordeaux University, Villenave d'Ornon, France) with or without the *S. cerevisiae* strain (Zymaflore FX10®, from Laffort, France, GN and SB[6]) were able to produce ATHP and ETHP. The impact of *B. bruxellensis* on ATHP and ETHP production (Figure 1) has already been shown and discussed in detail by Moulis *et al.* (2023)[2].

TABLE 1. Composition of 1 L for N-Heterocycles Assay Medium (*N*HAM).

Component	For 1 L
Ammonium citrate tribasic	2 g
Calcium chloride	1 g
Citric acid	2 g
D-Fructose	50 g
Ethanal	100 mg
Ethanol (96 %)	52 mL
Iron sulphate	43 mg
L-Lysine	5 g
L-Ornithine	5 g
Magnesium sulphate	12.5 mg
Malic acid	5 g
Manganese sulphate	25 mg
Potassium chloride	4.25 g
Potassium phosphate monobasic	5.5 g
Tween 80	1 mL
pH	4.5

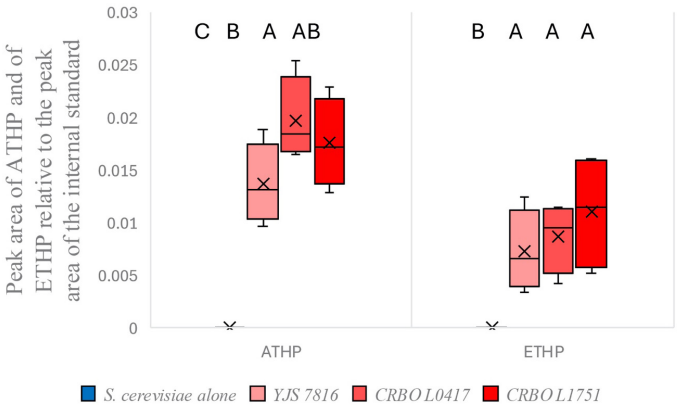


FIGURE 1. Comparison of ATHP and ETHP produced by 3 strains of *Saccharomyces cerevisiae* as a function of co-inoculated strains of *Brettanomyces bruxellensis* (n=6, ANOVA p-value < 0.05).

When *B. bruxellensis* and *S. cerevisiae* strains are co-inoculated, most of the differences in the results are due to *B. bruxellensis*, which is responsible for 88 % of the changes in ATHP levels and 62 % in ETHP levels. Variations in the results are also due to S. cerevisiae strains, albeit to a lesser extent (Figure 2). Figure 3 displays a boxplot of ATHP and ETHP produced by the three *B. bruxellensis* strains depending on the *S. cerevisiae* strain. The letters accompanying each boxplot summarise the Tukey post-hoc tests for the two compounds, indicating the significant differences observed. For ATHP, production varied among the added *S. cerevisiae strains*. The *B. bruxellensis* strains produced more when inoculated with SB than with FX10. The production of ETHP by B. bruxellensis increased when inoculated with both SB and GN.

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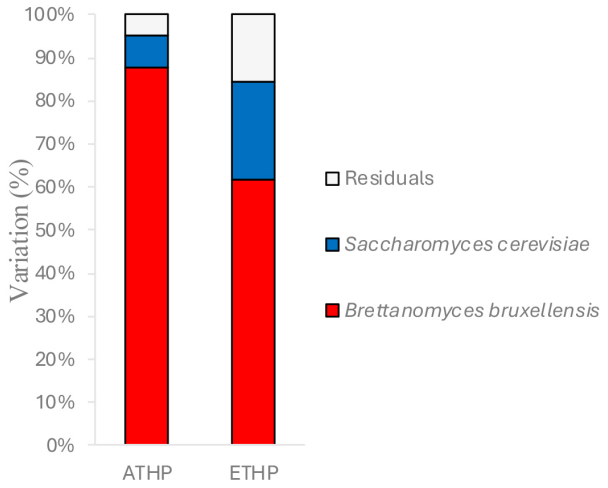


FIGURE 2. Variability explained (p-value < 0.05) for each species.

Differences in the production of acetaldehyde have been observed among various strains of *S. cerevisiae*[7] [8]. Acetaldehyde is identified as a precursor of mousy compounds[5] [9], prompting an investigation into its presence in NHAM after incubation with different *S. cerevisiae* strains.

Initially, acetaldehyde is introduced into the NHAM at a concentration of 100 mg/L. The levels of acetaldehyde after incubation with *S. cerevisiae* are detailed in Table 2. The FX10 and GN strains appear to consume the acetaldehyde in the medium, whereas the SB strain seems to increase in concentration. This could account for the previously observed synergistic effect of the SB strain, although the behaviour of *S. cerevisiae* strains might change entirely in the presence of another microorganism. However, this does not account for the ETHP production observed when GN is present.

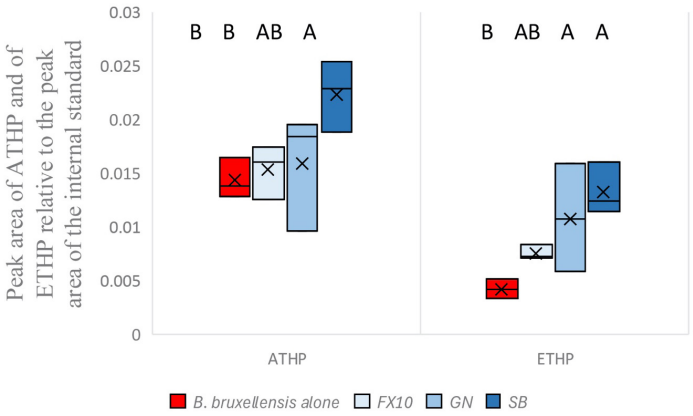


FIGURE 3. Comparison of ATHP and ETHP produced by 3 strains of *Brettanomyces bruxellensis* as a function of co-inoculated strains of *Saccharomyces cerevisiae* (n=6, ANOVA p-value < 0.05).

TABLE 2. Acetaldehyde levels after the incubation, in NHAM, of three strains of *Saccharomyces cerevisiae* (FX10, GN, SB).

	Acetaldehyde (mg/L)	
	Av[a]	SD[a]
FX10	50	6
GN	77	8
SB	114	8

a: Av, average of the microbial duplicates; SD, standard deviation of the microbial duplicates.

Another factor that might account for the interaction between *S. cerevisiae* and other microorganisms is the accumulation of lees at the bottom of the tubes, which inadvertently introduces additional nutrients and amino acids into the environment. These substances may be utilised by the organisms responsible for producing mousy compounds. Ultimately, each microorganism may contribute individually to the metabolism involved in generating these compounds.

Conclusion

There appears to be a significant interaction between *S. cerevisiae* and *B. bruxellensis* when co-cultured in the same sample. The presence of specific strains of S. cerevisiae in the environment may also affect the development of mousy off-aromas by other microorganisms. This study is the first to examine the interactions between various microorganisms in relation to the production of mousy compounds, which until now had only been tested in monoculture. Species once thought to be non-producers might actually contribute to the complex effects of a consortium, suggesting that alcoholic fermentation and the specific yeasts used could influence the production of these compounds by other microorganisms. While these findings provide new insights into microbial interactions during fermentation, they remain preliminary regarding their potential impact on industry practices. Further research is needed to confirm these effects and to explore their implications for fermentation management and quality control. ■

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