

A new assay for the screening of brewing yeasts in their ability to excrete hydrogen sulfide

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SUMMARY

Hydrogen sulfide plays a key role in beer quality: I) consumers can easily detect its rotten-egg typical aroma II) it can easily bind to allylic alcohols to generate polyfunctional thiols. Polyfunctional thiols are known to have a strong impact on the overall aroma of many fermented foods. In some beers, they could also impart some strong defects like onion-flavours (1). In order to help brewers to avoid unpleasant flavours in beer, a new assay initially proposed to screen other genus of yeasts (2) was used to investigate a large series of *S. cerevisiae* and *S. pastorianus* strains issued from our yeast collection. Closed plate flasks containing a zinc-agar layer above the liquid microbial culture are proposed as a trap system where the H₂S can be retained and then quantified by the methylene blue reaction.

INTRODUCTION

Hydrogen sulfide has been tentatively quantified thanks to a large panel of methods, including electrochemical, chromatographic and spectroscopic procedures. The methylene blue reaction is the most common approach for spectroscopic detection (3). Few methods have been developed for the direct determination of H₂S production by microorganisms. Generally, H₂S is trapped in alkaline solutions (pH >10) separated from the culture medium. Other methods, using specific membrane on the plate (4) have also been developed.

The interest of this new method is the double layer system. The trapping medium is in the same plate as the culture medium but does not influence the microorganism fermentation.

of zinc acetate and 4,5 ml of NaOH to 100 ml of agar. The solution was then stirred until complete homogeneity. Ten millilitres of this solution was then immediately poured into each flask. They were then left horizontally until solidification in a sterile hood.

Methylene blue reaction

The H₂S produced during fermentation at 28 °C by yeast strains is trapped in the zinc-agar layer (figure 2a). After 1 or 9 day(s), the medium is removed, the flask turned down (figure 2b) and the H₂S liberated is quantified *in situ* using the methylene blue reaction (figure 1). 1 ml of 4-amino-N,N-dimethylaniline (58 mM in HCl 6M) and 0,2 ml of iron chloride (23 mM in HCl 1,2 M) were added on the agar layer and left for 20 min.

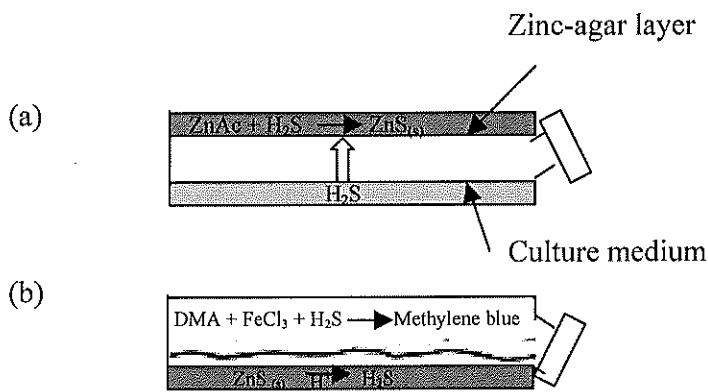


Figure 2: (a) Trap system for the capture of the H₂S and (b) quantification by the methylene blue synthesis reaction.

In this assay, the pH balance is used to trap and release the hydrogen sulfide (figure 3).

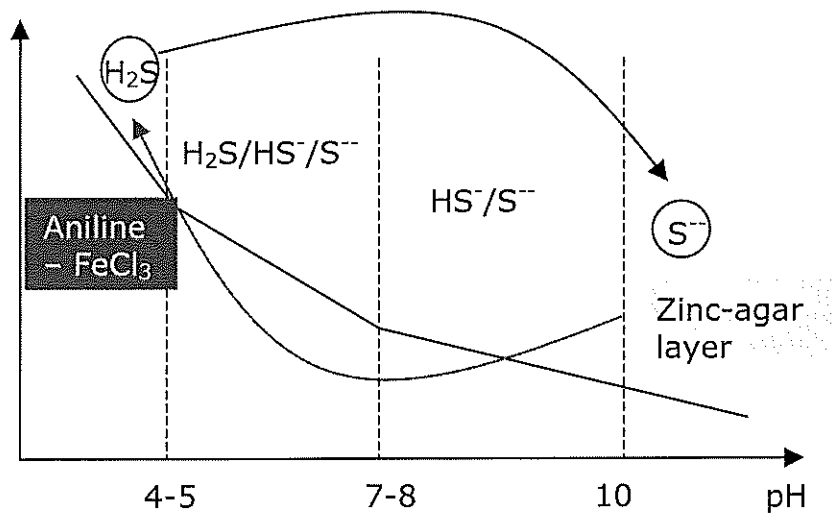


Figure 3: Relationship between pH and ionic form of sulfur.

According to our results, the limit of detection was 0,2 $\mu\text{g H}_2\text{S/ml}$ (6 μM) and is in agreement with the limit of detection observed by Lopez del Castillo Lozano *et al.* (2). The maximum standard deviation observed was 2,9 %. Standard deviation was obtained with the Spooled value (Spooled = $(A^{-1} * \Sigma\sigma^2)^{1/2}$ where A = number of measures, σ = standard deviation. Other methods recorded lower detection limits but with a restricted range of analyses and a maximum concentration fixed at 100 μM . This assay was first applied to screen different kinds of yeast strains used for the bakery (BkY), the wine (WY) and the brewery industry (bottom-fermentation (BY) and top-fermentation (TY)). The amount of H_2S was measured after 1 and 9 days of fermentation (figures 5 a and b).

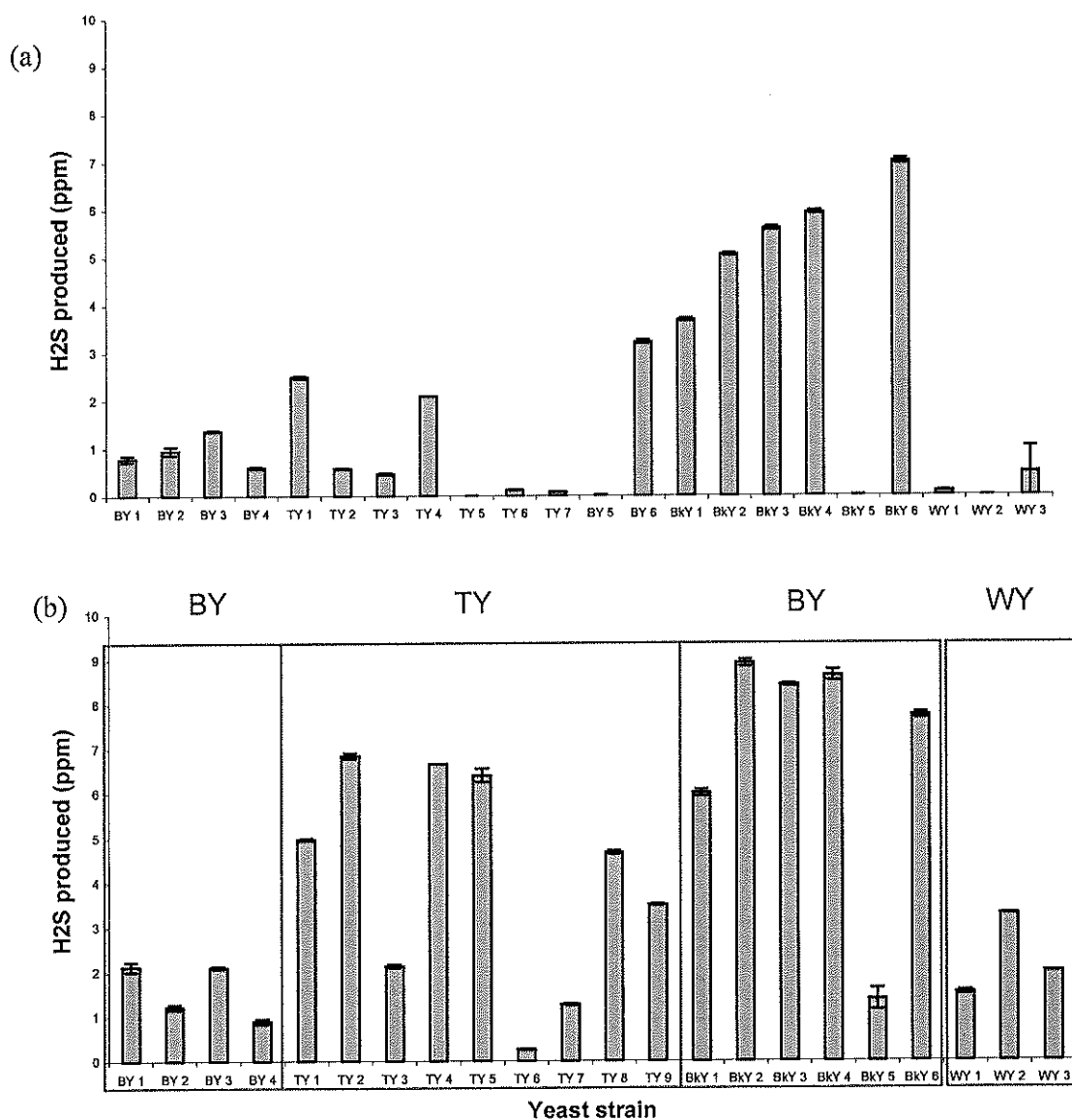


Figure 5: Amount of H_2S produced by each studied yeast strain (bottom fermentation (BY), top fermentation (TY) (*S. cerevisiae*), bakery (BY) (*S. cerevisiae*) and wine (WY) yeasts) after 1 (a) and 9 (b) days of fermentation in closed plate flasks.

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