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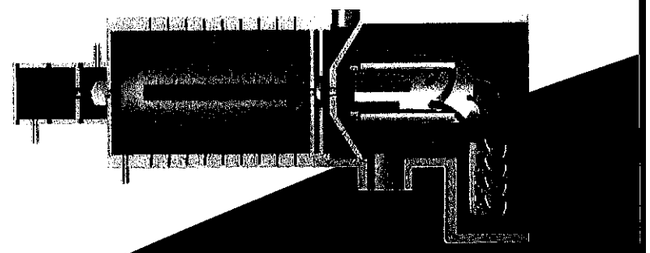

FastGC PTR-ToF-MS analysis of yeast VOCs

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Abstract

For the first time in this study proton-transfer-reaction time-of-flight mass spectrometry (PTR-ToF-MS) equipped with a prototype fast-GC system and a multipurpose head-space automated sampler was used to investigate variability between four meiotic segregants of M28 natural *Saccharomyces cerevisiae* strains and two mating type of BY strains of *S. cerevisiae* strains during their growing in a rapid and non-invasive way. The technique was successful in characterizing selected yeast strains.

Introduction

Yeast metabolism plays a key role in the production of flavor compounds in alcoholic beverages thus affecting their final quality and sensory profile. Volatile compound concentration is influenced by the growth characteristics of yeast strains. For this reason a rapid and non-invasive screening of the yeast volatilome is of outmost relevance. Proton-transfer-reaction time-of-flight mass spectrometry (PTR-ToF-MS) showed promising results in monitoring *Saccharomyces cerevisiae* volatile production in dough and bread (Makhoul et al. 2014). However, this technique does not allow separating structural and spatial isomers that complicates the identification and quantification of the compounds. Furthermore due to high ethanol production during fermentation processes PTR-ToF-MS parameters should be changed drastically or an inert gas should be introduced in order to prevent hydronium ion depletion and the generation of ethanol dimers and trimers, clusters between ethanol and water, and their fragments. In the present work a new approach is proposed for the analysis of yeast volatile organic compound (VOCs) by the coupling of PTR-ToF-MS both to a prototype fastGC system and a multipurpose head-space automated sampler. In this case the chromatographic separation provided by fastGC permits to discriminate compounds with the same chemical formula as well as to eliminate the undesired effect of high ethanol concentration.

Experimental Methods

Four meiotic segregants of the M28 natural *Saccharomyces cerevisiae* strain (M28-1A, M28-1B, M28-1C and M28-1D) and two BY *S. cerevisiae* strains (BY4741 – MAT *a* and BY4742 – MAT *α*) were selected for studying their VOCs profiles by a commercial PTR-ToF-MS 8000 instrument (Ionicon Analytik GmbH, Innsbruck, Austria) coupled both to a multipurpose head-space automated sampler (Gerstel GmbH, Mulheim am Ruhr, Germany) and a prototype fastGC system (Ionicon Analytik GmbH, Innsbruck, Austria) with a short polar GC column (MXT-WAX Cap. Column 6m, 0.25mm ID, 0.25um, Restek, Bellefonte, PA). The samples (twelve biological replicates of each yeast strain, the substrate used for their growth (solid YPD, 1% Yeast Extract, 2% Peptone, 2% Dextrose) and lab air) were left for 12 days in dark at 30°C with regular aeration. The headspace of each sample was measured at seventh and twelfth day of the experiment for 15 seconds which guaranteed total replacement of headspace by pure air. The injection time to fastGC sample loop was set to 4 seconds which was enough for its complete filling. The fastGC temperature ramp was from 40°C till 220°C and lasted 130 seconds, which was optimal for the separation of the investigated VOCs.

Data processing of PTR-ToF-MS spectra consisted of dead time correction, external calibration and peak extraction (Cappellin et al. 2010). Retention time shift has been accounted for by aligning the chromatogram to the peak of oxygen (O_2+, m/z 31.989).

Results and discussion

The preliminary analysis of fastGC chromatograms showed the possibility to distinguish between every M28 meiotic segregants and BY strains in the size of the chromatographic peaks (i.e. m/z 41.038, 43.017, 43.054, 57.069, 61.028, 71.085, 89.059, and others). In general BY strains produced more VOCs than M28 ones. In particular BY4742 showed higher total VOC emissions than BY4741 one. M28-1A and 1C colonies were more active VOC emitters than other two M28 ones.

The comparison of the results of two time-points demonstrated the significant decrease of ethanol and other VOC emissions by all yeast colonies, especially in M28-1B and M28-1D colonies, which can be explained by different growth characteristics and resistance to metabolic starving [3]. However the production of methanol (m/z 33.033) and acetone (m/z 59.049) augmented for all yeast samples during second time-point measurement most probably due to aging of yeast colonies.

In this work, for the first time, PTR-ToF-MS coupled both to a multipurpose headspace automated sampler and a prototype fastGC system was applied for a rapid and non-invasive analysis of the yeast colonies. The technique was successful in characterizing different yeast strains and identifying differences in the release of important classes of compounds.

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