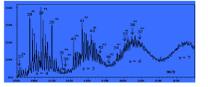


# In vivo mass spectrometric analysis of yeast growth metabolism



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## 1. Introduction

The diversity of volatile organic compounds (VOCs) produced by yeast (*Saccharomyces cerevisiae*) is enormous. Many of these compounds are still unknown.

We present a real time non invasive technique to measure metabolites in the mass range 50-500 Da with a time resolution of 1 min, 24 hours a day in autonomous operation.

We have used and experimentally validated this technique for the study of yeast metabolism from lag phase to exponential growth and stationary phase.

## 3. Results

In the first stage of the experiments we feed yeast (*Saccharomyces cerevisiae*) with <sup>13</sup>C<sub>6</sub>-glucose and follow Ethanol and Acetic acid signal. Yeast growth is monitor in real time using a time lapse camera.

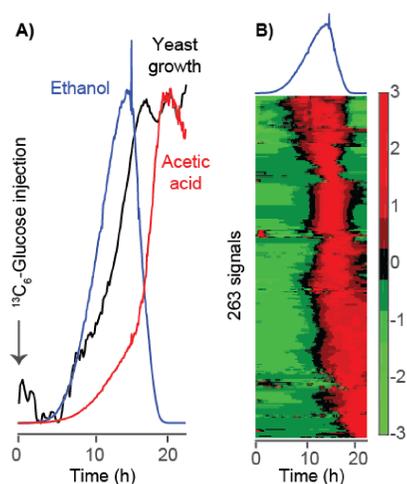


Figure 1A shows the normalized time profiles of ethanol, acetic acid and relative yeast grown.

Figure 1B shows a heat map with 263 interesting metabolites detected. Many of them were previously unknown for yeast.

In the second stage, we fed WT, ZWF1 and PFK1 with <sup>13</sup>C<sub>1</sub>-glucose with the aim of identifying differences in the metabolism of the three yeast strains.

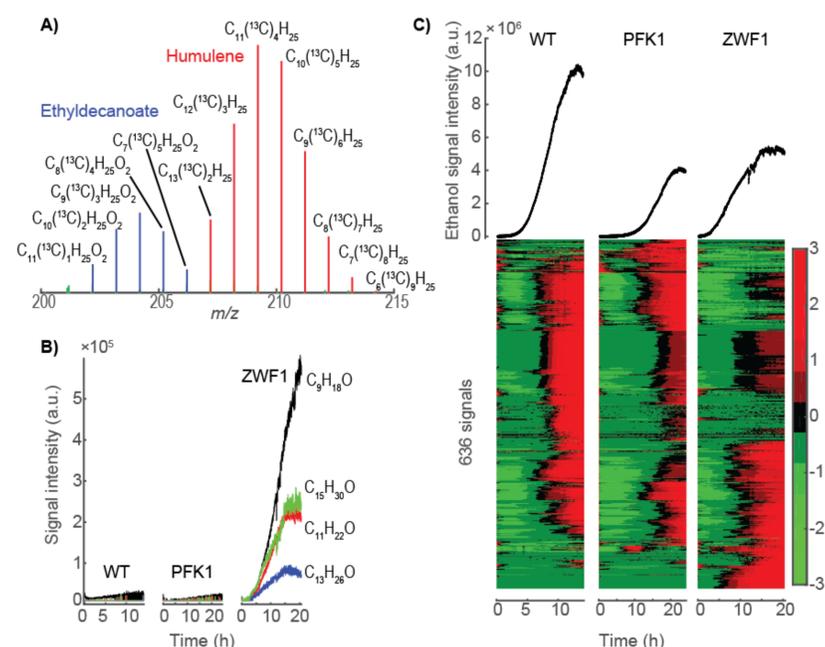


Fig. 2A shows a typical example of the mass spectra acquired during such measurements. It shows the average spectrum for WT during ~15 h of growing.

Fig. 2B shows some metabolites that appeared only in ZWF1.

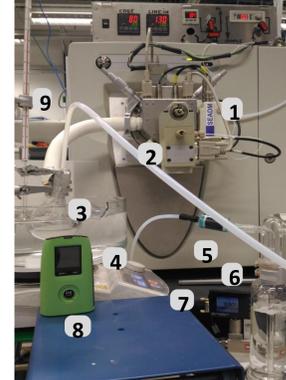
Fig. 2C shows that Ethanol production is much higher in WT than in PFK1 or ZWF1 as it is expected.

The heatmap shows an overview of all the signals detected

## 2. Methods and Materials

All experiments were conducted on an LTQ Orbitrap using a low Flow Secondary Electro Spray Ionization (SESI) designed for conducting Ambient MS in ETHZ. Thanks to its stability and efficiency we can run experiments for hours detecting slow changes in concentrations.

Our set up consist on the following material:



1. Mass Spectrometer
2. Low Flow-SESI
3. Bioreactor: Three-Neck Bottle 100mL, Caps for sealing and Teflon magnet
4. Magnetic stirrers at 800rpm, hot plate at 40 °C.
5. Gas Washing Bottles for humidifying the air.
6. Air Filter
7. Flow mass controller for constant flow of air at 0.5 lpm.
8. Lapse time camera for recording relative values of OD in real time
9. Glass thermometer

## 4. Identification

In a final stage, we look for the most metabolites according to their PCA and try to identify them

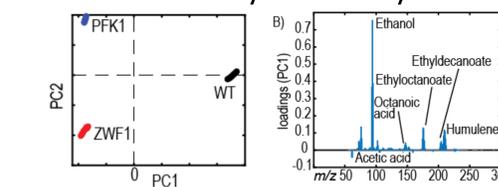


Fig. 3A shows a PCA score plot for the three strains during exponential growth, showing a clear separation for the three yeast strains.

Fig 3B shows that, along with ethanol acetic acid, a number of compounds contributed to the separation shown in this direction.

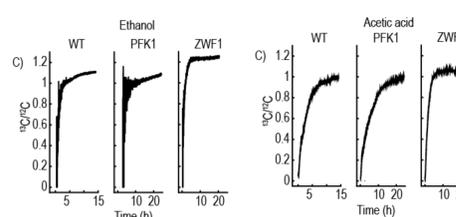
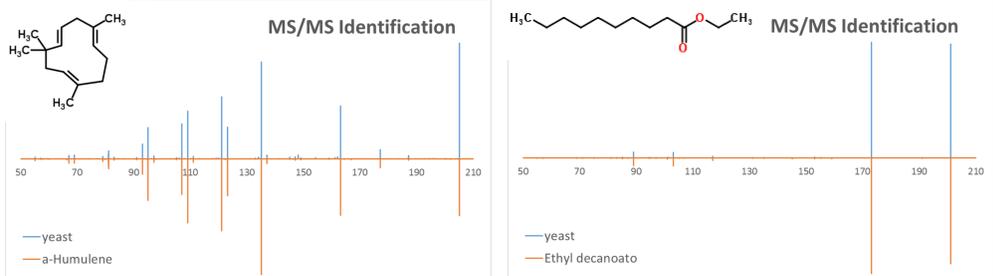


Fig 3C shows the 13C/12C ratio for ethanol (dimer) and acetic acid. Both the shape and ratios are different for WT, ZWF1 and PFK1 showing different kinetics.



## 5. Conclusion

We are now able to detect fast changes in concentrations of yeast metabolites occurred in the range of seconds after a glucose injection, but also slow changes that takes hours.

141 metabolites were detected simultaneously during yeast growth during 21 hours.

## 7. Acknowledgements

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## 8. References

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