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Compositional and ultrastructural characterization of the SMA strain of *Saccharomyces pastorianus*

Potter, G.[1,4], Swart, C. W.[1,2], Van Wyk, P. W. J.[2], Swart, H. C.[3], Budge, S. M.[4], Speers, R. A.[4]

[1]Department of Microbial, Biochemical and Food Biotechnology; [2]Laboratory for Microscopy; [3]Department of Physics, University of the Free State, Bloemfontein, South Africa.
[4]Canadian Institute of Fermentation Technology, Dalhousie University, Halifax, Canada. Correspondence: SwartCW@ufs.ac.za

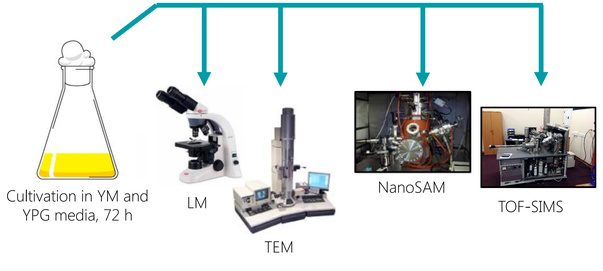
INTRODUCTION

It is well-known that carbon dioxide (CO_2) and ethanol are released as by-products during brewing fermentations, yet intracellular CO_2 was never observed in yeast cells until the development of Auger-architectomics (Swart et al., 2012). This discovery represented a paradigm shift in current models of intracellular gas generation, transport and cellular metabolism. In materials science a comprehensive compositional survey of a substance is only possible when surface analysis is combined with depth profiling. Fortunately, the field has developed several nanotechnological instruments to achieve such a complete approach including both Nano scanning Auger microscopy (NanoSAM) and time-of-flight secondary ion mass spectrometry (TOF-SIMS).

AIMS

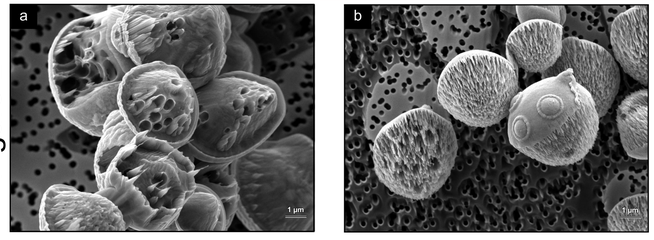
In this study the standard SMA strain of *Saccharomyces pastorianus* was analyzed with Auger-architectomics and TOF-SIMS (i) to further demonstrate the nascent biological applications of these techniques and (ii) to investigate the influence of bubble formation on cellular composition in fermenting yeasts. Nanotechnological analyses were coupled with microscopic examination via transmission electron microscopy (TEM) and high resolution (Hi-Res) scanning electron microscopy (SEM). For the purposes of comparison, cells were grown in fermentable yeast-malt (YM) and non-fermentable yeast extract-peptone glycerol (YPG) media.

MATERIALS AND METHODS



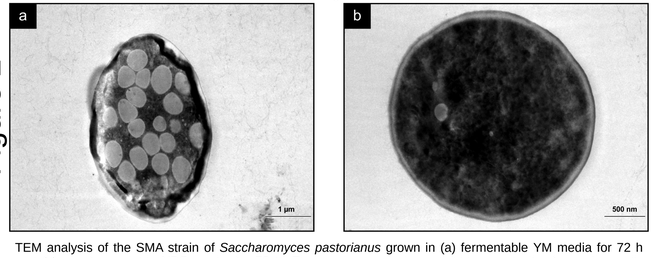
RESULTS

Figure 1



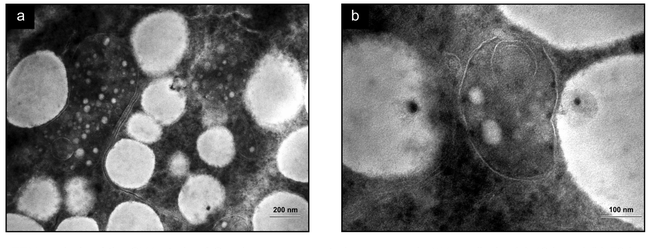
Post-Ar⁺ etching (36 mins) Hi-Res SEM analysis of the SMA strain of *Saccharomyces pastorianus* grown in (a) fermentable YM media (48h) and (b) non-fermentable YPG media (48h). YM-grown cells contained a large number of hole-like structures (gas bubbles), while YPG-grown cells contained significantly fewer bubbles.

Figure 2



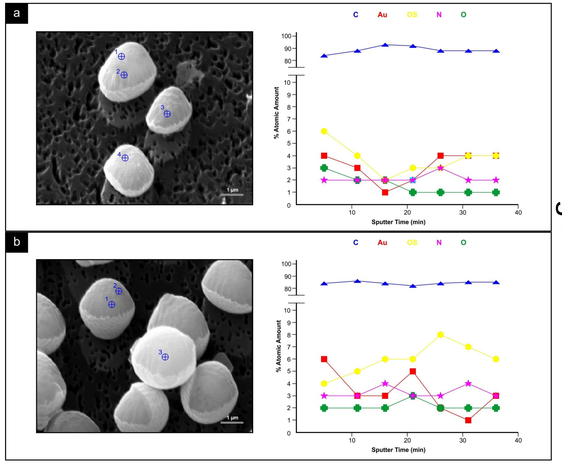
TEM analysis of the SMA strain of *Saccharomyces pastorianus* grown in (a) fermentable YM media for 72 h and (b) non-fermentable YPG media for 72 h. Electron-transparent gas bubbles inside the fermenting cells increased with fermentation time from 24 h to 72 h and the 72 h aged cells were almost completely filled. Pronounced dark-staining, lipid-rich osmiophilic layers also appeared as fermentation progressed. Cells grown in non-fermentable media contained few electron-transparent gas bubbles.

Figure 3



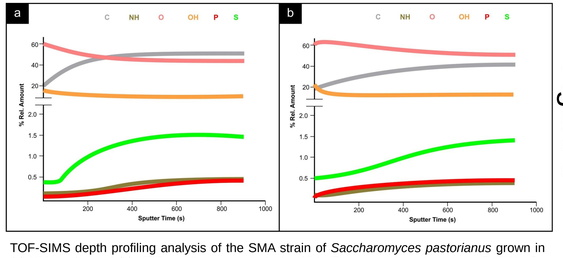
TEM analysis of the SMA strain of *Saccharomyces pastorianus* grown in YM media for (a) 48 h and (b) 72 h. Smaller localized networks of bubbles are visible in both the 48 h and 72 h cells. Even though Auger-architectomics has revealed the presence of intracellular bubbles in yeast, the origin of the bubbles inside the cells remains a mystery. These smaller networks of bubbles, which seem to be centralized in certain regions of the cell, provide some of the first clues as to where bubbles originate in fermenting yeasts.

Figure 4



NanoSAM SEM and Auger electron spectroscopy analysis of the SMA strain of *Saccharomyces pastorianus* grown in (a) YM and (b) YPG media for 24 h. SEM micrograph of (a) YM 24 h cells and (b) YPG 24 h cells. The targets for elemental analysis are shown by the circles and cross circles. A graph of elemental analysis as % atomic concentration relative to sputtering for target 3 from YM 24 h cells and target 2 from YPG 24 h cells is also shown next to the corresponding micrograph. % Relative atomic concentrations of C, N, O, Au and Os in both YM and YPG cultures varied with sputtering time from the same defined location in the cell.

Figure 5



TOF-SIMS depth profiling analysis of the SMA strain of *Saccharomyces pastorianus* grown in (a) YM media and (b) YPG media respectively for 48 h. The negative atomic ions C^-, NH^-, O^-, OH^-, P^- and S^- were tracked. The intensities were normalized at each sputtering time and are expressed as % relative amounts.

DISCUSSION

Hi-res SEM indicated an increase in the number of gas bubbles with fermentation time (24 h – 72 h, YM fermentable media) (Fig. 1a, 48 h). YPG-grown cells (non-fermentable media) contained larger vacuolar structures and very few bubbles could be observed at any sampling time (Fig. 1b, 48 h). TEM results indicate a number of electron-transparent gas bubbles inside the fermenting cells that increased with fermentation time to a point where the 72 h aged cells were almost completely filled (Fig. 2a). The intracellular structures were not enveloped by a membrane suggesting that they were indeed bubbles and not cell organelles. Cells grown in non-fermentable media contained few electron-transparent gas bubbles (Fig. 2b). 72 h old cells (Fig. 2a) were filled with gas bubbles to a greater extent than any other *Saccharomyces cerevisiae* or *pastorianus* fermentation strains tested with the same TEM technique (Swart et al., 2012). TOF-SIMS depth profiling analysis showed that the cell compositions changed with sputtering time, and differed at the exterior and interior of the cell. C^- levels were highest and O^- were lowest in 48 h fermenting cells (Fig. 5a). C^- and O^- were the most abundant ions and these converged as sputtering time increased. O^- was much more abundant than NH^-, and P^- and S^- could be detected in both cell types, with greater amounts of S^- than P^- present. The prevalence of S^- over P^- may indicate a greater abundance of sulfur-containing amino acids relative to phosphorus-containing cellular lipids.

CONCLUSIONS

NanoSAM and TOF-SIMS applied to biological specimens represent powerful techniques to investigate the influence of bubble formation on cellular composition in fermenting yeasts. From TOF-SIMS depth profiling analysis it was clear that the cell exterior and interior had different elemental compositions. Nanotechnological analyses coupled with microscopic examination via TEM and Hi-Res SEM also revealed complex networks of intracellular bubbles that were present in fermenting but not respiring yeasts. Further use of these techniques should reveal how and where bubbles originate in brewing yeasts..

MAIN REFERENCES

Swart, C. W., Dithebe, K., Pohl, C. H., Swart, H. C., Coetsee, E., Van Wyk, P. W. J., Swarts, J. C., Lodolo, E. J. & Kock, J. L. F. (2012). Gas bubble formation in the cytoplasm of a fermenting yeast. *FEMS Yeast Res* **12**, 867–869.

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