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Intracellular gas bubble formation by the genus *Saccharomyces* is related to gas release

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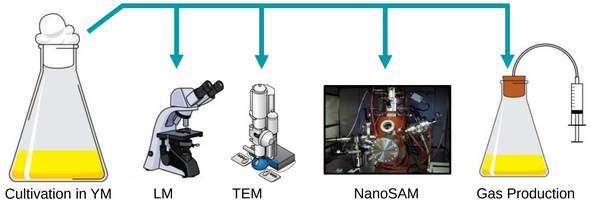
INTRODUCTION

Yeast of the genus *Saccharomyces* lies at the heart of many fermentation processes due to their ability to ferment with a high efficiency, however strains not used in commercial fermentation processes are thought to ferment with a lower efficiency. It is well-known that carbon dioxide (CO_2) and ethanol are released as by-products during fermentation, though it was not until recently that CO_2 bubbles were observed in the cytoplasm of *Saccharomyces cerevisiae* and *S. pastorianus*. Swart and co-workers (2012) discovered accumulation of large electron transparent structures in yeast cells of the genus *Saccharomyces* when they ferment, using Transmission Electron Microscopy (TEM). These structures were labelled as gas bubbles due to the lack of a surrounding membrane, which is characteristic of cell organelles. The content of the gas bubbles was verified by adding the metal salt, zinc sulfate ($ZnSO_4$), to the growth medium. The bubbles seemed to be galvanized when viewed with Nano Scanning Auger Microscopy (NanoSAM) and this can be ascribed to the reaction of zinc with carbonic acid (formed in a reaction between CO_2 and water) on the periphery of these bubbles to form insoluble or weakly soluble zinc bicarbonate.

AIMS

To determine the conserved status of gas bubble formation in the genus *Saccharomyces* and to correlate the number of these bubbles with the fermentation efficiencies of seventeen different strains, including commercial and non-commercial strains. Non-commercial strains are expected to ferment with a lower efficiency than commercial strains.

MATERIALS AND METHODS

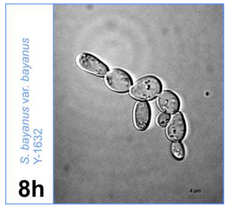


Cultivation in YM and YPG media, 25°C, 48 h | LM | TEM | NanoSAM | Gas Production

RESULTS

LM images after 8 h of growth (grown in fermentable media)

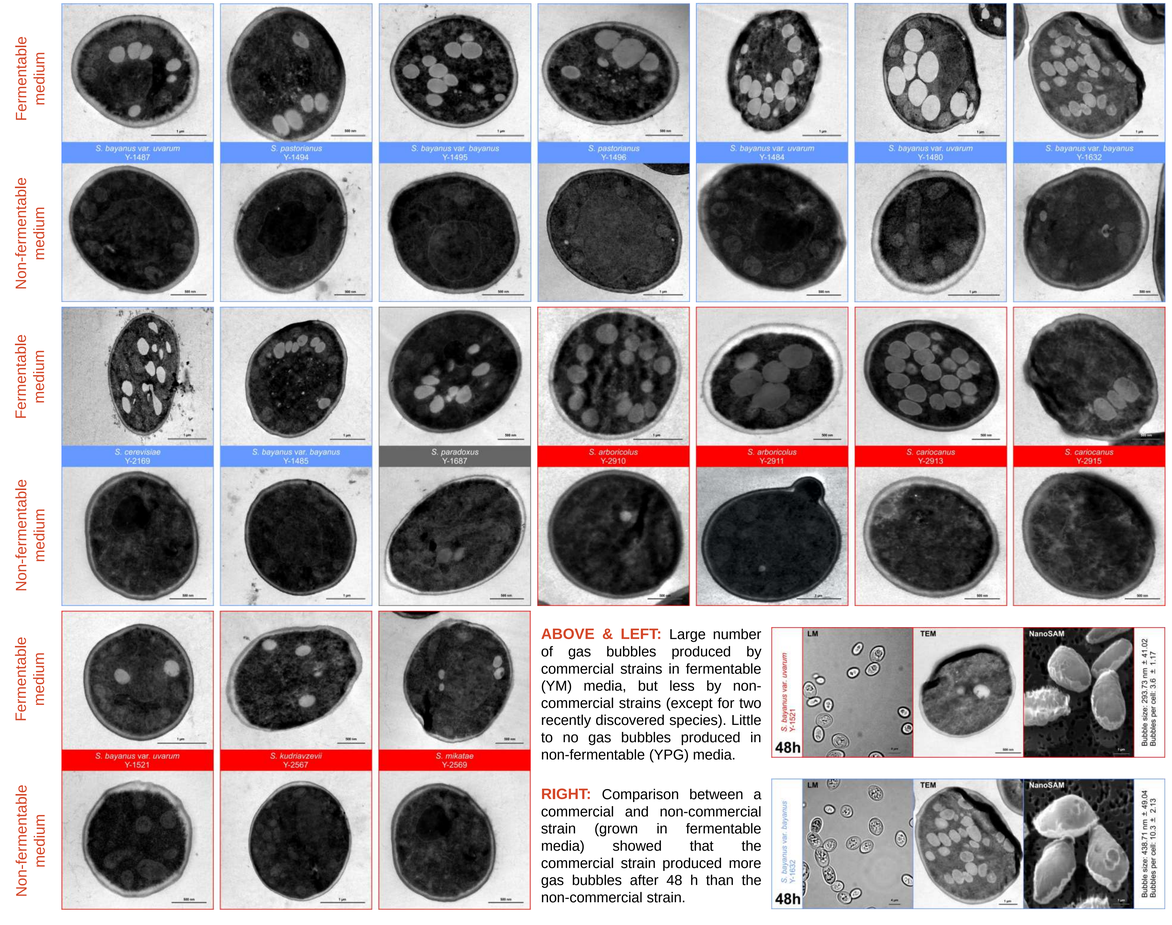
Increased number of gas bubbles observed in the non-commercial strain when compared to the commercial strain after 8 h of growth - consistent with fermentation profile (see table).



RESULTS (cont.)

Gas bubble formation (TEM)

Blue = Commercial | Red = Non-commercial | Grey = Unknown



ABOVE & LEFT: Large number of gas bubbles produced by commercial strains in fermentable (YM) media, but less by non-commercial strains (except for two recently discovered species). Little to no gas bubbles produced in non-fermentable (YPG) media.

RIGHT: Comparison between a commercial and non-commercial strain (grown in fermentable media) showed that the commercial strain produced more gas bubbles after 48 h than the non-commercial strain.

RESULTS (cont.)

Gas production assay

Strain	7h	7.5h	8h	8.5h	9h	9.5h	10h	10.5h	11h	11.5h	12h	12.5h	13h
Y-1521 (Caddisfly)	START			FULL									
Y-1484 (Pear juice)			START							FULL			
Y-2567 (Leaf)						START				FULL			
Y-1632 (Turbid beer)						START						FULL	
Y-2169 (Ale beer)							START						FULL

Fermentation profiles indicate rapid fermentation after 8 h of growth for a non-commercial strain, with longer time needed for gas production by commercial strains.

DISCUSSION

Gas bubbles produced during fermentation accumulated in all of the cells throughout the genus, in commercial and non-commercial strains alike. However, strains not used in commercial processes yielded fewer gas bubbles after 48 h of growth than strains used in commercial processes, except for two recently discovered species. Results from the gas production assay showed that a non-commercial strain filled the syringes entirely at about 8 h after inoculation before a commercial strain of the same species started to fill the syringes with gas. Subsequently, LM analysis after 8 h of growth showed that the non-commercial strain accumulated more bubbles than the commercial strain in contrast to growth after 48 h. This suggested a correlation between gas bubble formation and gas production.

CONCLUSIONS

It was found that non-commercial strains are not necessarily slow fermenters, but that a different fermentation profile might render them unfit for commercial processes, however this should be further investigated using more sensitive techniques to determine the volume of gas released and verify the number of bubbles present.

MAIN REFERENCE

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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