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# Characterization of the yeast gene YDL218W: Carbon source-dependent growth rates and cell wall defects

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## Background

***Saccharomyces cerevisiae***

- The budding yeast *Saccharomyces cerevisiae* is a common model organism for studying metazoan cell biology. It thrives in warm, fermentable-carbon-rich environments, and divides rapidly. The yeast genome was the first eukaryotic genome to be entirely sequenced. There are approximately 6000 yeast open reading frames (genes) and more than 1000 code for proteins with unknown functions.

**Yeast ORFan Gene Project**

- We are one of more than 30 research labs from primarily undergraduate research institutions (PUIs) around the country participating in an NSF study to characterize open reading frames of unknown function (called ORFans).

**YDL218W**

- All the ORFs in *S. cerevisiae* have been deleted and deletion mutants are commercially available. Our research focused on the ORFan called YDL218W, and we obtained a deletion mutant (*YDL218Δ*) to analyze and compare to the wild type. While little is known about YDL218W, there is some evidence to suggest it is involved in the formation or maintenance of the yeast cell wall, a structure of particular interest due to implications for future development of human fungal infection treatment among other uses.

**Evidence of Cell Wall Involvement**

1. The gene Azf1 is known to regulate transcription of genes, including YDL218W, that maintain *S. cerevisiae* cell wall integrity in non-fermentable carbon sources
2. YDL218W contains a MARVEL domain, and MARVEL-domain containing proteins are often related to membrane events in the cell wall (2).
3. YDL218W is upregulated when yeast is exposed to patulin (3), a mycotoxin found in rotting fruit that harms yeast cells (4). The capacity to resist patulin is correlated with increased 1, 3-β-glucan content in the cell wall (4), suggesting that upregulation of YDL218W plays a role in maintaining cell wall stability and resisting the effects of patulin.

**Experiments**

- We compared the growth of *YDL218Δ* and our wildtype through a colony formation assay on agar plates and by generating growth curves. We did both experiments in different carbon environments (fermentable and non-fermentable) to test our mutant's ability to grow in poor conditions.
- We also focused more specifically on the cell wall. The cell wall of *S. cerevisiae* consists of three primary components: 1, 3-β-glucan, mannoproteins, and chitin. Certain dyes can bind to these specific components and be analyzed using microscopy. Trypan Blue binds to and stains chitin and 1, 3-β-glucans (5). Chitin forms a ring on the surface of the cell (chitin ring) when it buds into a daughter cell, leaving behind a bud scare. We stained *YDL218Δ* and our wild type with Trypan Blue to qualitatively and quantitatively compare chitin concentrations using fluorescence microscopy.

## Literature Cited


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(2) Sánchez-Pulido, L., Martín-Belmonte, F., Valencia, A., Alonso, M. A. MARVEL: a conserved domain involved in membrane apposition events. Trends in Biochemical Sciences. 2002; 27(12): 599-601.
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## Objectives

We aimed to qualitatively and quantitatively assess differences in *YDL218Δ* compared to wild type. Specifically, our goals were to:

- Compare growth patterns and growth rates between our wild type and mutant in different carbon sources to both observe differences in our mutant and to test the effect of varying carbon environments that would introduce stress
- Observe the cell wall structure of both wild type and mutant. In particular, to compare chitin deposition at the budding point of rapidly dividing cells

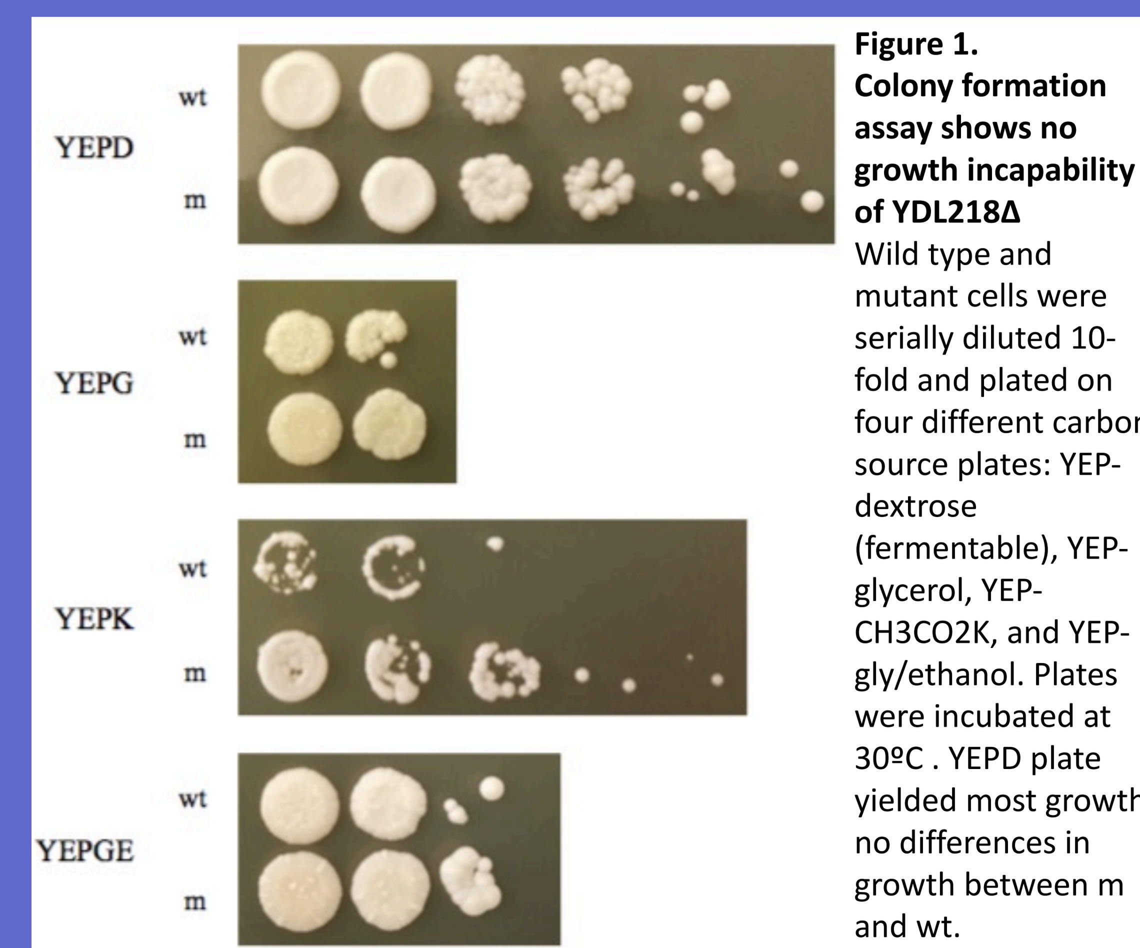



**Figure 1. Colony formation assay shows no growth incapability of YDL218Δ**
Wild type and mutant cells were serially diluted 10-fold and plated on four different carbon-source plates: YEP-dextrose (fermentable), YEP-glycerol, YEP-CH3CO2K, and YEP-gly/ethanol. Plates were incubated at 30ºC . YEPD plate yielded most growth, no differences in growth between m and wt.

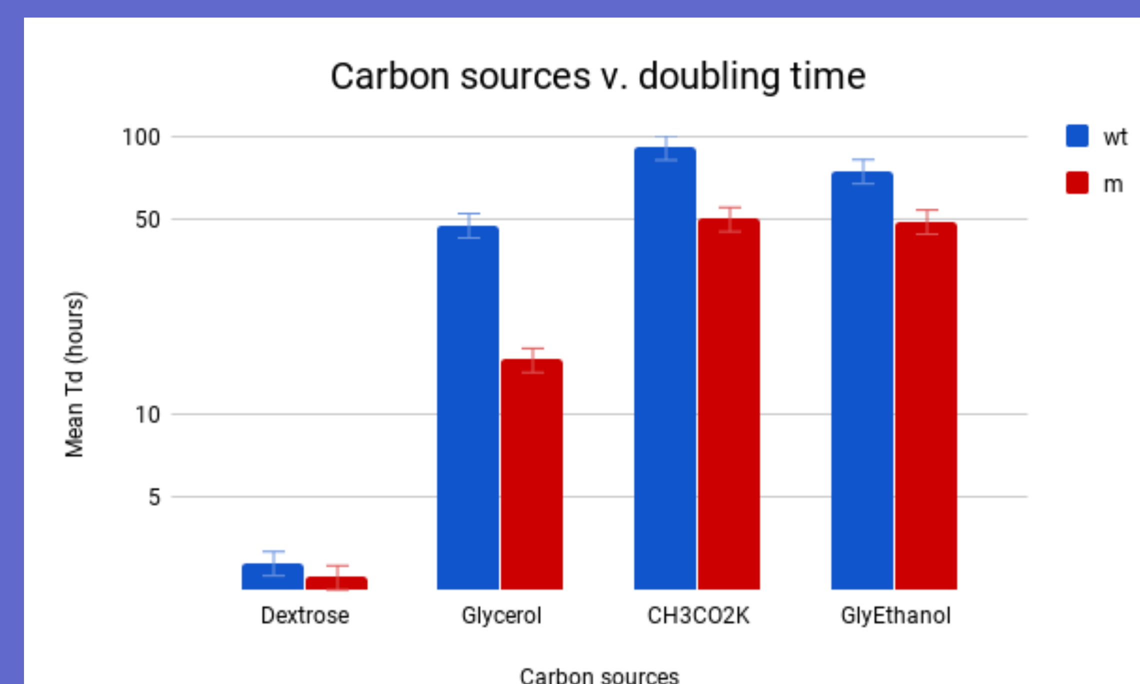
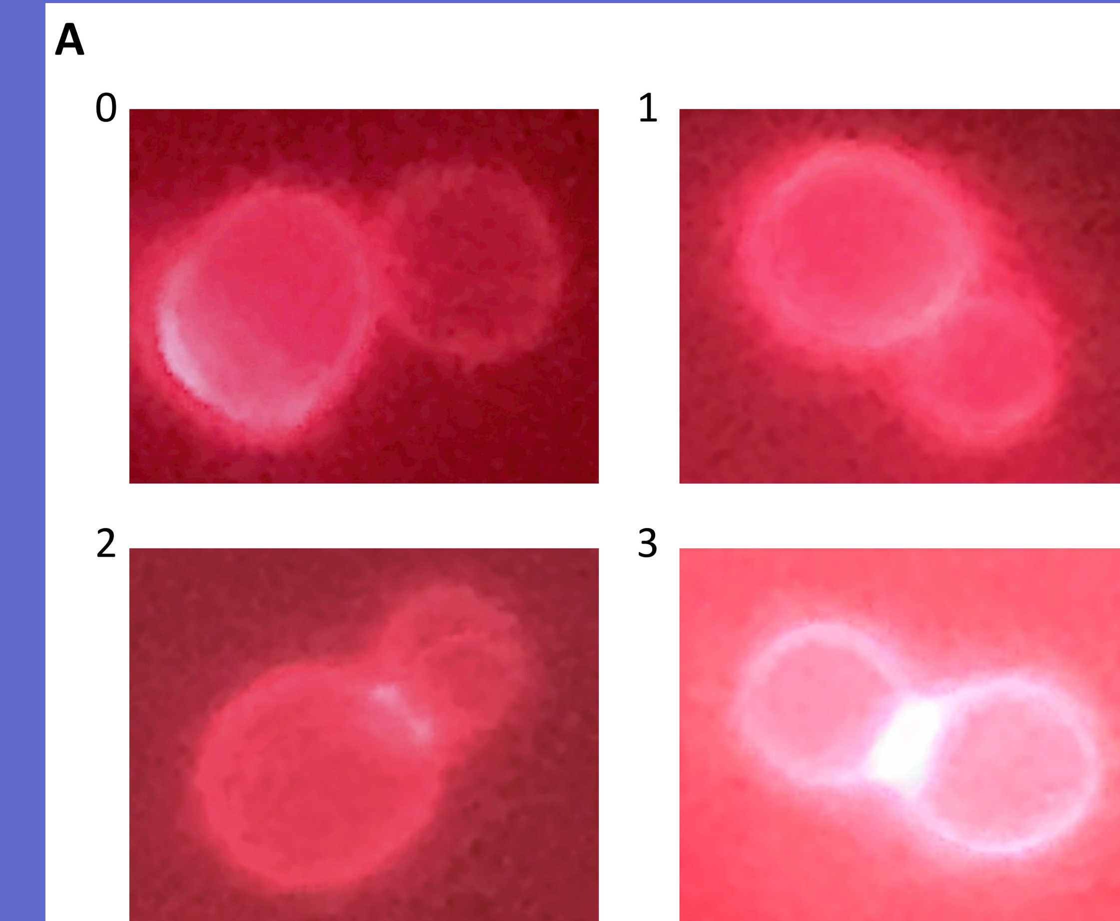



**Figure 2. Growth rate comparison shows *YDL218Δ* consistently grows faster among different carbon sources**
10μl of yeast starter culture was combined with 200μl of respective liquid media in a microplate and absorbency was read every 15 mins. for 40 hours. Growth rate curves were generated from microplate reader, and selected curves were used to calculate mean doubling times for mutant and wild type in each carbon source. Cells grew the fastest in the fermentable dextrose plate. Overall, *YDL218Δ* appeared to grow faster than the wild type in all carbon sources.

## Fluorescence Microscopy



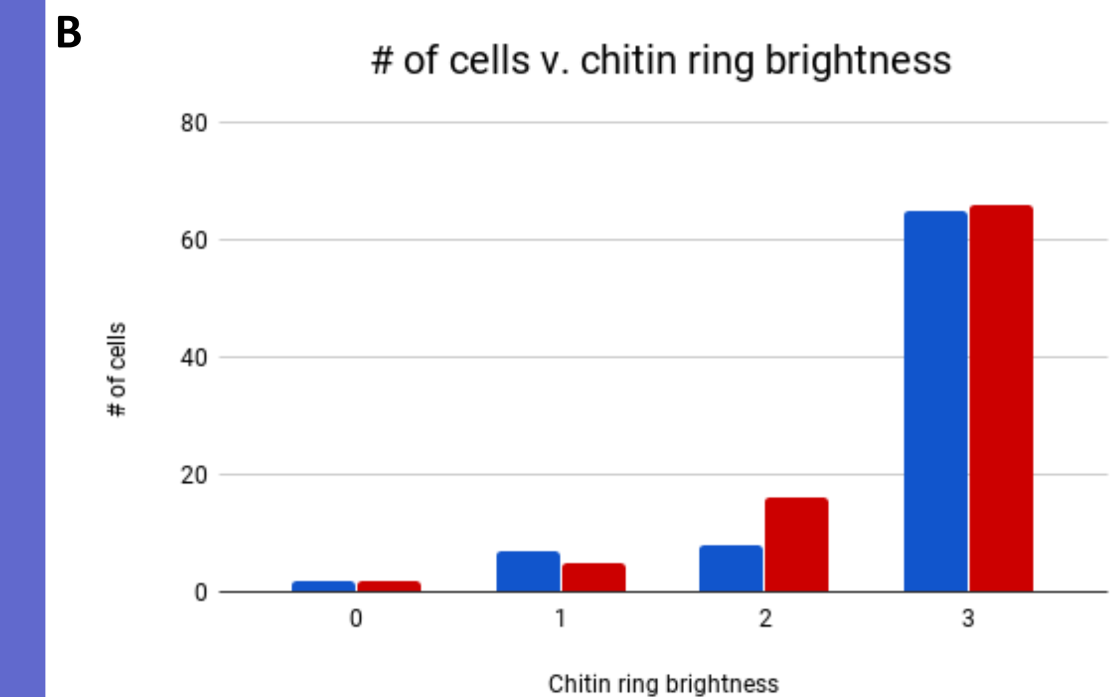




**Figure 3: Chitin deposition scoring, and comparison between mutant and wildtype show no difference in rate of deposition in YDL218Δ**
**(A).** Mutant and wild type cells were gathered by centrifugation for 2 mins. at 900rcf, re-suspended in phosphate buffer saline, and Trypan Blue (0.5mg/ml) was added (5). Cells were observed using fluorescence microscopy, and images were taken of budding cells. Chitin deposition was observed through the images, and scored using the following numerical system: 0=no visible ring, 1=some staining above background, 2=clear staining, 3=clear, bright staining. Examples of each are shown. (B). Scores for each level of chitin brightness were added and graphed to compare the wildtype to YDL218Δ. Most of the budded cells for both show high levels of chitin deposition at the bud point, indicated by a score of 3.

## Conclusions and Next Steps

**Conclusions**

- *YDL218Δ* appears to show no reduced growth capability compared to the wild type; in fact, our mutant may even have enhanced growth ability. This may mean YDL218W's primary function deals with another area of the cell.
- Staining of Trypan Blue showed increased chitin concentration around bud point. No obvious differences were found between mutant and wild type.

**Next Steps**

- Subjecting our wild type and mutant cells to chemical or physical stressors, and then use dyes to note changes in cell wall integrity. Stressors may cause a measurable difference in cell wall and related structures in the mutant.
- Expanding our quantitative analysis of the cell wall through electron microscopy. These images will be useful in offering a detailed picture of the cell wall, giving us the ability to locate minute differences in our mutant.