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Characterization of the Yeast Gene YDL218W: A Role in Cell Wall Biosynthesis and Maintenance?

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Characterization of the yeast gene YDL218W: A role in cell wall biosynthesis and maintenance?

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Introduction

Background

The budding yeast *Saccharomyces cerevisiae* is a common model organism used to study eukaryotic cell and molecular biology. *S. cerevisiae* is a fungus with a cell wall that makes it an excellent model for the study of antifungal agents (1). YDL218W is a yeast protein of unknown function. However, previous findings suggest that it may play a role in cell wall synthesis and/or maintenance.

Previous Findings:

- The gene AZF1 is known to transcribe a number of genes important for maintaining cell wall integrity. One of the targets of AZF1 is YDL218W.
- YDL218W contains a MARVEL domain, a domain commonly found in proteins involved in cell wall biosynthesis (2).

Importance

The number of fatal fungi related diseases in humans are becoming more prevalent all around the world (3). This increase in fatal fungi related cases is in part due to fungi becoming resistant to the few antifungal drugs available. As this becomes more of a problem, there is a need for effective antifungal drugs.

Objectives

Our primary objective is to characterize and the function of YDL218W through various experiments. Through various experiments, including:

- obtaining images of both mutant and wild type cells using Scanning Electron Microscopy (SEM)
- exposing both mutant and wild type cells to various cell wall disruptors and measuring effects on growth rates and cell lysis rates

Experiments

1. Comparison of wild type and mutant morphology using SEM.

Fix cells in progressively higher concentrations of ethanol, dehydrate using critical point drying, gold coat, and observe using OWU SEM.

2. Compare the effects of cell wall disrupter PHMB on growth rate of wild type and mutant cells.

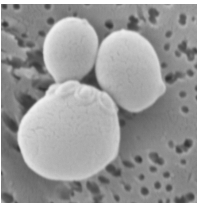
Dilute log phase cells into growth media plus PHMB and record OD₆₀₀ every ten minutes for 24 hours.

3. Compare the effects of the enzyme zymolyase on cell lysis rates between wild type and mutant cells

Dilute log phase cells into zymolyase solution and record OD₆₀₀ every ten minutes for 13 hours.

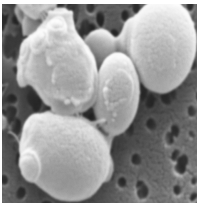
Results and Discussion

Experiment 1-WT



In image one to the left is a scanning electron microscopy (SEM) image of our wild type yeast cell. The bud scars appear normal, and cell division looks to have occurred normal as well. The cell wall appears smooth.

Experiment 1-mutant

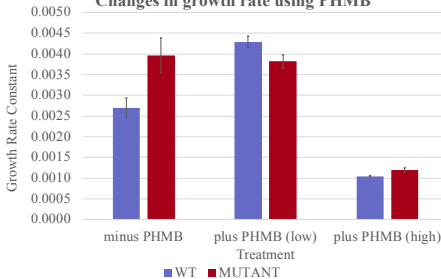


The SEM image to the left (Image 2) is of a mutant yeast cell with our deleted gene of interest. Compared to the wild type, the surface of the cell looks to be less smooth and the bud scars also look to be less consistent in size.

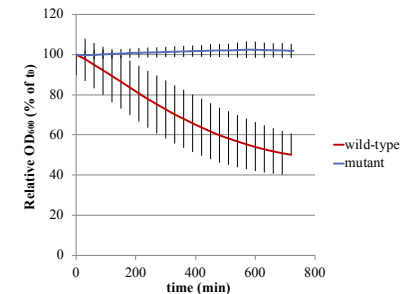
PHMB is an antifungal agent that is known to slow the growth rate of *S. cerevisiae*. We then hypothesized that with increasing concentrations of PHMB, growth rates would slow down. Our hypothesis was not supported when growth rate increased in the wild type when adding a low level of PHMB. The mutant growth rate went down slightly when adding a low level of PHMB, but then fell drastically when adding a high concentration of PHMB. The same could be said for the wild type. Growth rate appears to be similar for both the wild type and mutant when PHMB is present.

Experiment 2

Changes in growth rate using PHMB



Experiment 3



The wild type cells clearly show lysis over time much faster than the mutant cells. As the relative OD drops, less and less cells are contained within the zymolyase solution. As seen in the graph to the left, half of the cells have died that were originally in the solution. Contrary to the wild type, the mutant cell seemed to have an increased tolerance, and cells even appeared to grow over time.

Conclusions

Experiment 1

The differences in the cell wall between the wild type and mutant support the hypothesis that YDL218W is involved in cell wall synthesis or maintenance. Furthermore, it shows that we are able to successfully capture SEM images of *S. cerevisiae* with the equipment at Ohio Wesleyan. This is important as SEM will become crucially important in future experiments.

Experiment 2

PHMB is a commercially available antifungal agent. In previous experiments, strains with deletions in cell wall genes were more sensitive to PHMB compared to wild type (4). We hypothesized if YDL218W is necessary for cell wall maintenance, the mutant would be more sensitive to PHMB. However, our results did not support this hypothesis. In repeating this experiment we will take a range of increasing PHMB concentrations. Also, testing for statistical significance would be useful to make the final conclusions.

Experiment 3

Zymolyase is an antifungal agent that targets a key component of the yeast cell wall. In previous experiments, only a few known genes when deleted are known to cause the cell to be more tolerant to zymolyase (4). We hypothesized that deleting YDL218W would increase tolerance to zymolyase, and our results supported that hypothesis. This leads us to the follow up experiment where we will look into the component that zymolyase attacks and determine how YDL218W is involved.

Going forward:

- Repeat growth curve experiment
- Utilize SEM in experiments with zymolyase
- Explore TEM experiments
- Work towards characterizing gene function using other cell wall disruptors.

Acknowledgements

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Literature cited

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