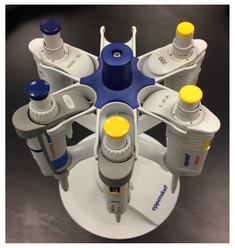

IRA2 gene's effects on *S.cerevisiae* growth

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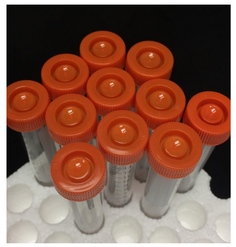


Introduction

In *Saccharomyces cerevisiae*, the Ras-cAMP pathway plays a role in the regulation of metabolism, stress resistance, and cell cycle progression.

Increased levels of cAMP are required for growth and cell cycle progression while decreased levels of cAMP result in high stress tolerance and arrest of growth and cell cycle.

Paper and microfluidic devices offer excellent, cost effective platforms to perform chemical analysis, and are valuable tools to analytical chemistry.

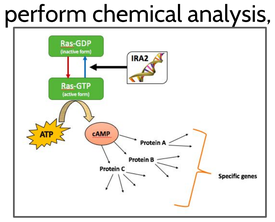


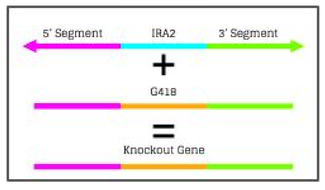
Figure 1. Process of Ras signaling

Figure 2. Original 3D assay

We hypothesize that if we knock out the IRA2 gene from the *Saccharomyces cerevisiae, then the mutant yeast will exhibit more growth and a lower resistance to stress. We also hypothesize that paper based platforms will be more effective than 3D platforms.*

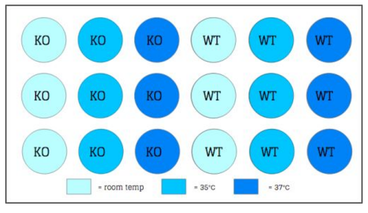
Methods

PCR



We knocked out the IRA2 gene with an antiobiotic resistance marker. The IRA2 gene was thus replaced with KAN (G418 antibiotic resistance) gene. This was done by finding primers 500 base pairs up stream and down stream of KAN on the *Saccharomyces* Genomic Database (SGD). These primers were the template for our PCR and amplified. We took advantage of the fact that the yeast experience a double strand break, they look for homologous DNA to repair the break. Therefore, when they experience this break, they take up the template DNA we amplified

Temperature



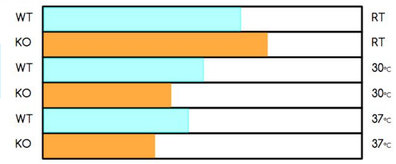
We conducted three different trials of each strain of yeast at three different temperatures (room temp, 35°C 37°C). Chart is color-coded to discern difference in temperature.

pH

	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH
WT	2.98	2.98	2.98	2.98	2.98	2.98	2.98	2.98	2.98	2.98	2.98	2.98
KO	2.98	2.98	2.98	2.98	2.98	2.98	2.98	2.98	2.98	2.98	2.98	2.98
WT	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
KO	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
WT	8.99	8.99	8.99	8.99	8.99	8.99	8.99	8.99	8.99	8.99	8.99	8.99
KO	8.99	8.99	8.99	8.99	8.99	8.99	8.99	8.99	8.99	8.99	8.99	8.99
WT	1/4	1/16	1/64	1/256	1/1024							
KO	1/4	1/16	1/64	1/256	1/1024							½ WT ½ KO

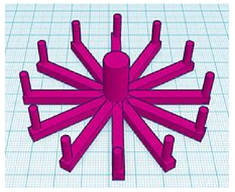
We grew the yeast in liquid Yeast Peptone Dextrose (YPD: Yeast extract 10g/L, Peptone 20g/L, and Dextrose 0.36g/L) at three different pH levels: pH 5 (optimal), pH 3 and pH 9 on 96 well plates. We cultured the WT and KO for each pH, for a total of six cultures in total. We used the spectrophotometer to measure optical density per 20 min and growth rate (OD/min) by finding an average of their steepest slopes of growth curves.

Paper Platform



The design intended to grow 3 of the wild-type and 3 of the IRA2 mutant strains in adjacent rectangular channels that are separated by wax barriers at 25 °C, 30 °C and 37 °C (Fig 1). By plating the yeast at the base of the channel, it is forced to grow in a projected and measurable direction as opposed to circularly, allowing for simple yet accurate visual and distance-based quantification of data.

3D Platform



This 3D platform was used to attempt to quantify the yeast growth of mutant and wild type yeast with and without the IRA2 gene. Each one of those arms will have a different pH environment inside and the yeast was inoculated in the center cylinder. The yeast then grows radially in whichever pH environment it will grow the best in. 1: There was no statistical difference in growth rate between the wild-type and IRA2 deficient strain at 25°C and 30°C. A T-test expressing a *p-value of 0.01336* for 37°C showed that the mutant strain statistically grew less than the wild-type.

Results

1. At 37°C, The IRA2- mutant strain grew less than the wild-type.

P value 0.77653824 1 0.01336183

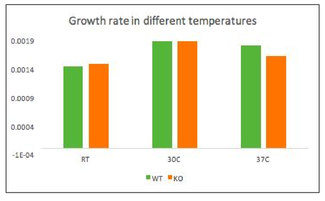


Figure 1. There was no statistical difference in growth rate between the wild-type and IRA2 deficient strain at 25°C and 30°C. A T-test expressing a *p-value of 0.01336* for 37°C showed that the mutant strain statistically grew less than the wild-type.

2. At pH 5, The IRA2- mutant strain grew less than the wild-type.

P value 0.57876955 0.01005971 0.085577658

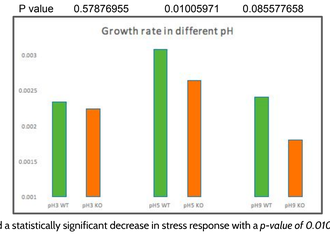
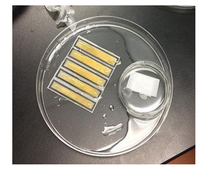


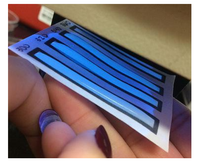
Figure 2. At pH 5, the IRA2- strain exhibited a statistically significant decrease in stress response with a *p-value of 0.010*. Both wild-type strains in pH 3 and pH 9 exhibited large variability among each of their 12 samples. Although the knock-out strain at pH 3 and pH 9 grew less than their wild-type counterparts they did not exhibit this in a statistically significant manner with large *p-values of 0.576* for pH 3 and *0.0856* for pH 9.

3. The paper-based assay for distance-based analysis was successful in design and unsuccessful in application.



3a

3b



3c

Figure 3. **a)** Our use of nail polish as a hydrophobic barrier was a great success, as was the use of a hot plate to reflow the wax on our PAD. Issues arose once the agar on the first print dried out. **b)** We attempted to remedy this issue by adding small pieces of wet paper towel to the petri dish containing the PAD which was not successful. Nor was the use of a smaller petri dish full of water within the original dish because it spilled over during transport and washed away the yeast from the agar, eliminating any results. The issue was finally solved by covering the smaller petri dish in Parafilm and poking holes in it. **c)** After optimizing channel width, we went through the agar optimization process. In the last agar optimization trial, the Green Fluorescent Protein did not fluoresce in a substantial manner, suggesting there was trouble growing the yeast.

4. The microfluidic device could not be completed.

4a

4b

Figure 4. **a)** The very first 3D print was broken before any testing could be done with it intact. However, it was used to try and create the first PDMS mold. During this process we found that the print was too large to fit in a standard petri dish so it was placed in a larger one. **b)** There was not enough PDMS to fill to the volume of the larger dish so folded up Parafilm was added to occupy more area and raise the level of PDMS This was unsuccessful and no mold was obtained. Once the scaled-down replacement was received, it was discovered that there was no more curant for the PDMS and no results could be obtained.

Conclusions

- The mutant strain of yeast may exhibit more growth and less resistance to stress than the wild type
- The paper and 3-D platforms were properly optimized
- The yeast exhibited no significant growth on the paper based platforms
- Nail polish acts as a good hydrophobic barrier that does not impair yeast growth

Acknowledgements

We would like to thank Dr. Van Dyken and Dr. Knecht for all of their support and insight throughout this process. Without them, none of this would have been possible. Additionally, we would like to acknowledge our teaching assistants Shuang Xu, Yiwen Ji, Lily Acheampomaa-Piasare and Reid Collis for guiding us throughout our investigation. Finally, a huge thanks to University of Miami for providing us with the facility, resources, and opportunity to conduct these experiments.