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# Analyzing the effect of benzylamine product on yeast cells deficient in the oxidative stress response

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## Abstract

Oxidative stress occurs when there is an imbalance of reactive oxygen species and antioxidants. The brain is especially vulnerable to the effects of ROS because of its high oxygen demand. Previous studies have demonstrated that oxidative stress plays a central role in common neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease. Furthermore, ROS have been found to trigger programmed cell death in cancer cells. Our lab aims to investigate the oxidative stress response in *Saccharomyces cerevisiae* by studying its growth at different concentrations of a compound called benzylamine, which is thought to have possible anticancer effects. To identify the different biological pathways being affected by the compound, we began by testing different concentrations of the compound with DMSO on yeast mutants. Each mutant had a different non-essential gene deleted. We found that the yeast has different growth compared to the control at both the 30nM and 300nM concentrations while still not being completely wiped out. After evaluating the growth patterns of twenty-six different yeast mutants in comparison to untreated and wild type cells, we identified four proteins to focus on that are involved in similar biological pathways: *Δzwf1, Δyap1, Δaif1*, and *Δtsa1*.

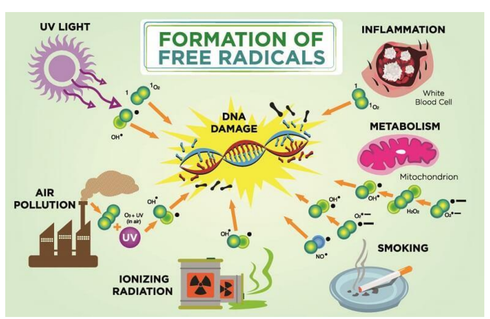


Fig 1. examples of both endogenous and exogenous sources of oxidative stress

## Methods

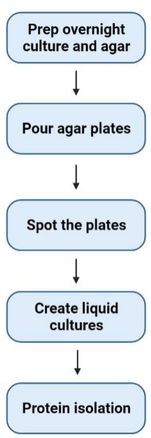


Fig 2. Flowchart showing the methodological steps leading up to protein isolation

## Results and Discussion

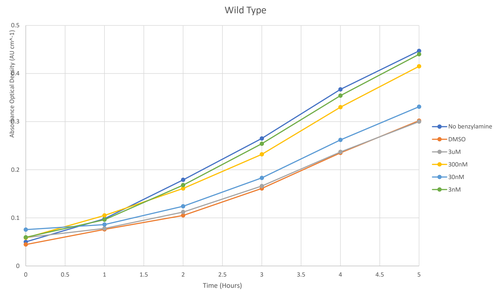


Fig 3. shows the change in optical density levels of wild type yeast cells when exposed to different concentrations of benzylamine

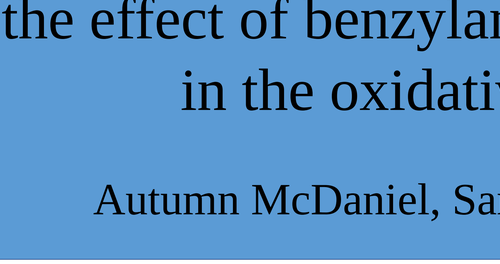


Fig 4. shows the change in optical density of *Δerg6* mutated yeast cells when exposed to different concentrations of benzylamine

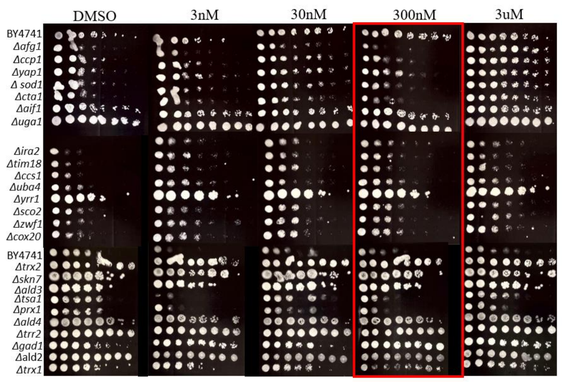


Fig 5. Shows the serial dilutions of the different yeast mutations at a high concentration of DMSO. Each mutation listed on the left are gene deletions. The 300nM concentration was used in the next step to see how benzylamine affects protein expression.

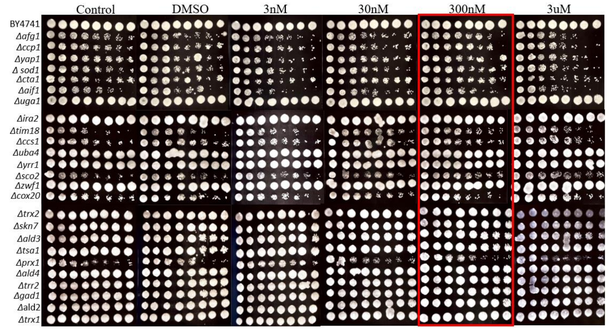


Fig 6. Shows the serial dilutions of the different yeast mutations at a low concentration of DMSO

| | DMSO | 3nM | 30nM | 300nM | 3uM |
|---|---|---|---|---|---|
| **BY4741** | 0 | 0 | 0 | 0 | 0 |
| ***Δafg1*** | - | - | - | - | - |
| ***Δccp1*** | 0 | - | - | - | - |
| ***Δyap1*** | - | - | - | - | - |
| ***Δsod1*** | 0 | - | - | 0 | - |
| ***Δcta1*** | 0 | 0 | + | 0 | - |
| ***Δaif1*** | 0 | - | + | 0 | - |
| ***Δuga1*** | 0 | 0 | 0 | 0 | 0 |
| ***Δira2*** | 0 | 0 | 0 | 0 | 0 |
| ***Δtim18*** | 0 | 0 | + | + | + |
| ***Δccs1*** | 0 | 0 | + | 0 | 0 |
| ***Δuba4*** | 0 | 0 | 0 | 0 | 0 |
| ***Δyrr1*** | 0 | 0 | 0 | 0 | 0 |
| ***Δsco2*** | 0 | + | + | + | + |
| ***Δzwf1*** | 0 | 0 | 0 | 0 | 0 |
| ***Δcox20*** | 0 | - | 0 | - | 0 |
| ***Δtrx2*** | 0 | 0 | 0 | 0 | 0 |
| ***Δskn7*** | 0 | 0 | 0 | 0 | 0 |
| ***Δald3*** | - | - | - | - | - |
| ***Δtsa1*** | - | 0 | - | 0 | - |
| ***Δprx1*** | - | + | 0 | 0 | 0 |
| ***Δald4*** | 0 | 0 | 0 | 0 | 0 |
| ***Δtrr2*** | 0 | 0 | 0 | 0 | 0 |
| ***Δgad1*** | 0 | 0 | 0 | 0 | 0 |
| ***Δald2*** | 0 | 0 | 0 | 0 | 0 |
| ***Δtrx1*** | 0 | 0 | 0 | 0 | 0 |

Table 1. Quantifies the cell growth from the spotted plates. A plus sign indicates that the growth has increased compared to the control, a minus indicates a decrease, and a 0 indicates no change compared to the control. Gene deletions outlined in red were chosen to study further in the next step of looking at the effect of benzylamine on protein expression

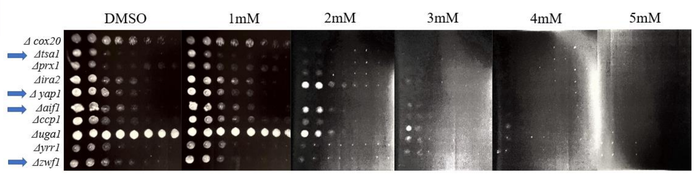


Fig 7. Shows serial dilutions of the combined effect of 30nM benzylamine concentration and hydrogen peroxide on cells

## Summary

- Benzylamine is able to enter the yeast cells with an intact membrane, indicating that it has the potential to affect cell growth and metabolism (Figures 3 and 4).
- The yeast mutants have different growth patterns at different concentrations of benzylamine. Some concentrations appear to be growth inhibitory while others promote growth.
- Hydrogen peroxide and benzylamine limited cell growth drastically at concentrations greater than 2mM. This indicates a potential interaction between oxidative stress pathways and benzylamine.
- *Δyap1, Δtsa, Δaif1*, and *Δzwf1* showed differences in growth and are involved in oxidative stress response

## Future Work

1.Further observation into specific cellular pathways impacted by benzylamine
2.Protein isolations
3.Western blots to determine the effect of benzylamine on the expression of proteins involved in the response to oxidative stress
4.Similar co-treatment tests in breast cancer cells to determine minimum growth inhibitory concentration when combined with oxidative stress promoter

## References

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