

## Introduction

-*Clostridium perfringens* is a pathogenic bacteria that is a major cause of exaggerated inflammation in the gut of broiler chickens, leading to a two-billion-dollar loss to the poultry industry annually. (Van Immerseel et al., 2009)

-Current techniques to treat infections by the bacteria use antibiotics, however, antibiotics can disrupt the native microbiota in the gut. A useful strategy to combat invading pathogens is to harness probiotics; one of the most widely used is *Saccharomyces boulardii*. The engineering of this yeast species to secrete antimicrobial factors can help treat pathogenic infections in the gut. (Cruz et al., 2022)

-However, these probiotics are limited in the number of protein factors that can be secreted. In this project, we engineered *S. boulardii* with an amylase gene and created mutants with UV mutagenesis. We then screened for mutants with improved protein production, which would be capable of producing increased amounts of antimicrobial factors.

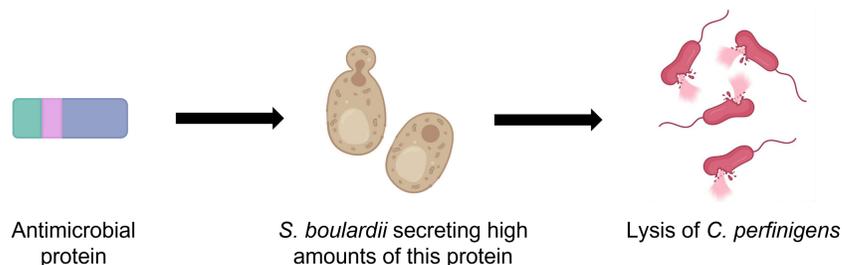


Figure 1: *S. boulardii* with improved secretory abilities can produce more antimicrobial proteins against pathogenic bacteria.

## Methods

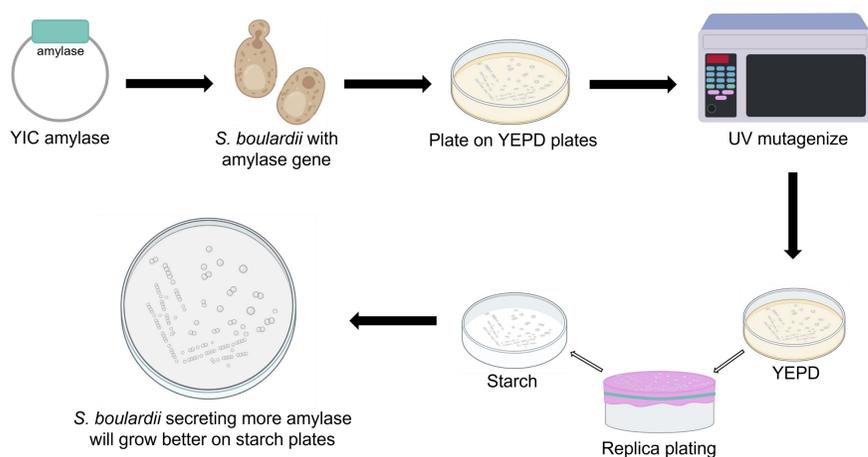


Figure 2: Design of *S. boulardii* with increased secretory properties. An amylase gene was ligated into a yeast integrating plasmid (YIC). This recombinant plasmid was transformed into the probiotic yeast *S. boulardii* through a lithium acetate protocol. Yeast containing the amylase gene was selected for and grown on yeast growth media (YEPD) plates. The yeast cells on the plates were mutagenized in a UV Stratalinker Box. The best secretors were selected and grown in YEPD media to lose their amylase gene. Select colonies were replica-patched on YEPD and minus tryptophan (-TRP) plates to identify cells without the amylase gene.

## Results

### Constructing an Integrating Amylase Gene for Secretion Screening

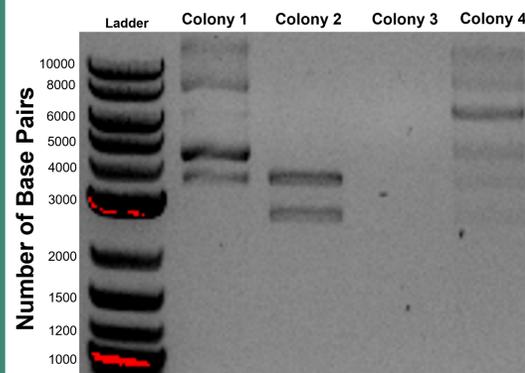


Figure 3: Double digestion to screen for clones with ligated YIC-amylase. PGK and YIC-amylase were double digested with the restriction enzymes XbaI and PstI. The isolated amylase gene was then inserted into the cut YIC vector. The recombinant plasmid was transformed into DH5a *E. coli* cells, these cells were then grown, and the plasmid was purified by a mini-prep protocol. The purified plasmids were then screened by double digestion with EcoRI and PstI to release two bands visualized by agarose gel electrophoresis with the amylase gene around 2741 bp and a 3649 bp vector.

### Screening for Amylase Activity in Transformed *S. boulardii*

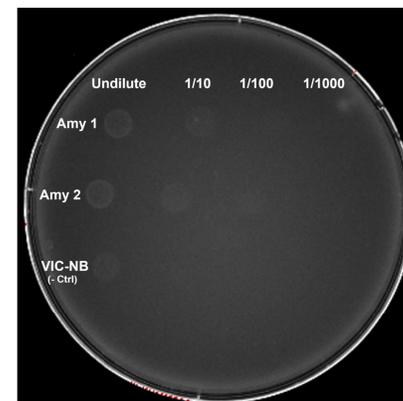


Figure 3: Evaluating Amylase Activity of *S. boulardii* Transformed with YIC Amylase. *S. boulardii* was transformed with the purified YIC-amylase recombinant plasmid by a lithium acetate transformation protocol. Colonies that appeared after two days were patched onto -TRP plates and grown in -TRP media overnight. Serial dilutions of the cultures were made and plated on starch plates to evaluate amylase activity.

### Killing Curve Assay for Mutagenized *S. boulardii*

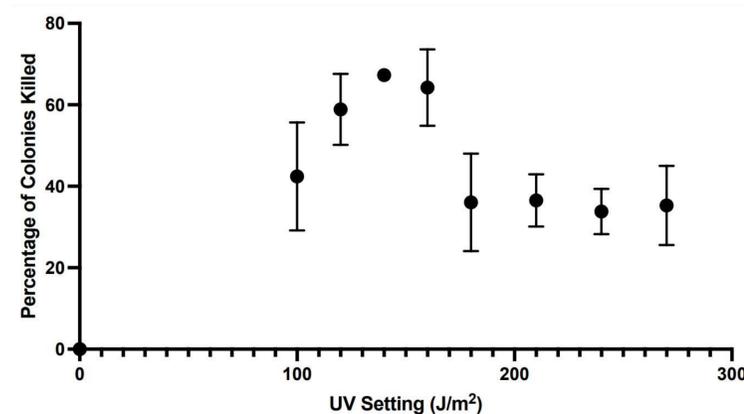


Figure 5: Killing Curve of Transformed *S. boulardii* Subjected to UV Mutagenesis. Selected *S. boulardii* transformed with YIC-amylase were plated on YEPD plates generating 300 colonies. The plates were subjected to different UV settings ranging from 100-270 J/m<sup>2</sup> in a UV Stratalinker Box. The plates were wrapped in foil and incubated for a day after which colonies that appeared were counted to quantify the number of cells killed by UV mutagenesis. This process was done to achieve 65% killing activity.

### UV Mutagenesis of Selected *S. boulardii*

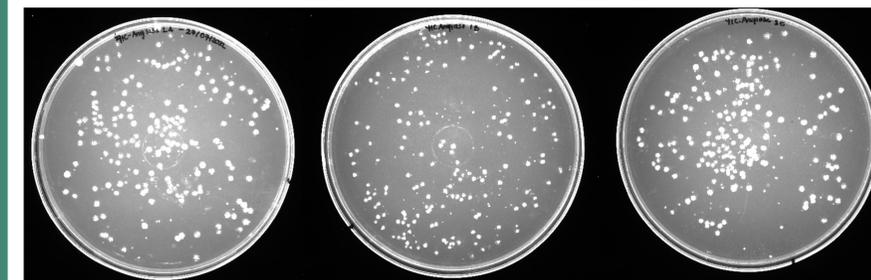


Figure 5: Plating of Selected Mutagenized *S. boulardii*. Selected *S. boulardii* transformed with YIC-amylase were mutagenized at a setting of 140 J/m<sup>2</sup>. The plates were wrapped in foil and colonies appeared after a day. These colonies were then replica plated onto starch plates and incubated at 30°C.

## Conclusions

-*S. boulardii* was successfully transformed with an integrated amylase expressing gene, these cells grew on starch plates indicating amylase secretion.

-Selected *S. boulardii* with this amylase gene was mutagenized with a killing efficiency of 45%.

-The surviving colonies were replica plated on starch plates and we successfully identified increased secretors from these plates.

-Yeast cells were grown multiple times in YEPD to allow them to naturally lose their YIC-amylase gene to allow for the integration of antimicrobial plasmids.

## Future Directions

Increased secretor yeast identified on the starch plates can be isolated and transformed with antimicrobial plasmids such as an endolysin gene that has been constructed to specifically target and kill *C. perfringens*.

## Acknowledgements

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## References

- Cruz, K. C. P., Enekegho, L. O., & Stuart, D. T. (2022). Bioengineered Probiotics: Synthetic Biology Can Provide Live Cell Therapeutics for the Treatment of Foodborne Diseases. *Frontiers in Bioengineering and Biotechnology*, 10, 890479.
- Van Immerseel, F., Rood, J. I., Moore, R. J., & Titball, R. W. (2009). Rethinking our understanding of the pathogenesis of necrotic enteritis in chickens. *Trends in Microbiology*, 17(1), 32–36.