

Abstract

Saccharomyces cerevisiae, commonly known as brewer's yeast, can be used as a powerful producer of many industrial materials. This project worked towards identifying the transporters in yeast responsible for the export of glucosides. Using glucosides with distinct coloration or fluorescence, ATPase inhibitor and gene knockout procedures can be performed to assess the ability of the export process in the yeast. Gene knockouts can be done by identifying prospective transporters in the cells through literature and using CRISPR to remove the corresponding genes. The cells and supernatants were analyzed using a spectrophotometer for presence of glucosides in and outside the cell. This information can aid in the industrial production of glucosides, and it can help provide information for future research using yeast cells.

Motivation

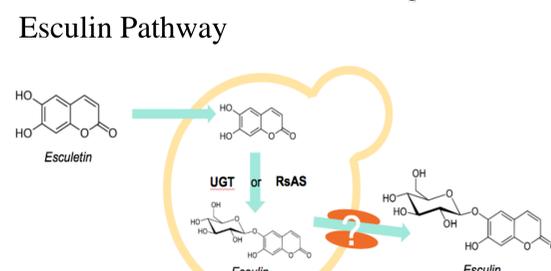
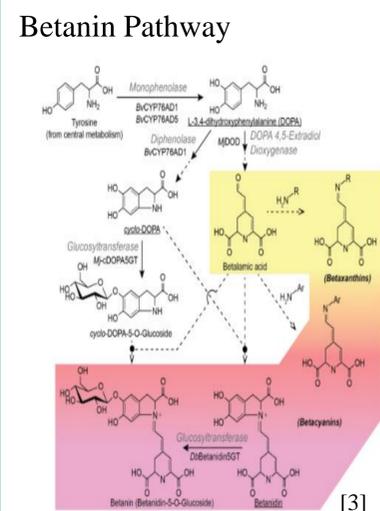
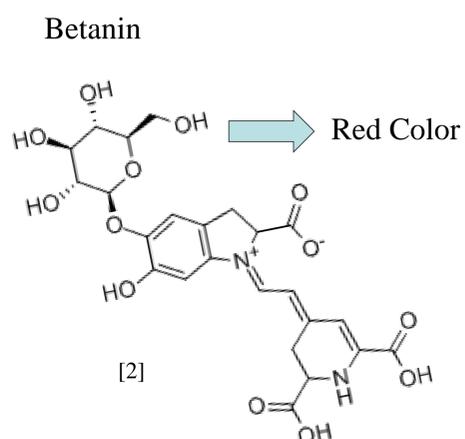
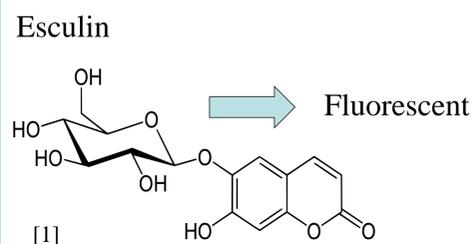
Using *Saccharomyces cerevisiae* cells, a glucoside pathway can be inserted to allow for production of glucosides.

- Glucosides are used for industrial purposes for clothing dyes and food flavorings.
- Currently, glucosides are made using environmentally intense and less efficient processes.
- Identification of transporters used in exportation of glucosides can be utilized to increase production of the industrial materials.

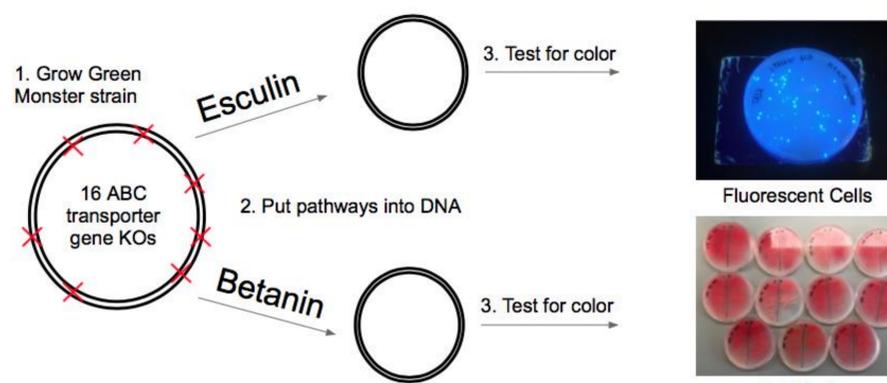
Introduction

The identification of the transporters in *Saccharomyces cerevisiae* used for glucoside transport involves a process of elimination to determine the source of the export.

- Green Monster is a strain that was engineered to remove 16 ABC transporters from the genes of *Saccharomyces cerevisiae*.



Methods



- Grew up Green Monster strain
- Transformed Esculin and Betanin pathways into the Green Monster DNA
- Grew up new strains in media for production assay
 - For Esculin, fed 1mM, 100µM, 10µM, and 0µM of Esculetin in SC-Leu
 - For Betanin, grown in minimal media
- Used TECAN and UV light to measure amount of color produced

Results

Esculin Results

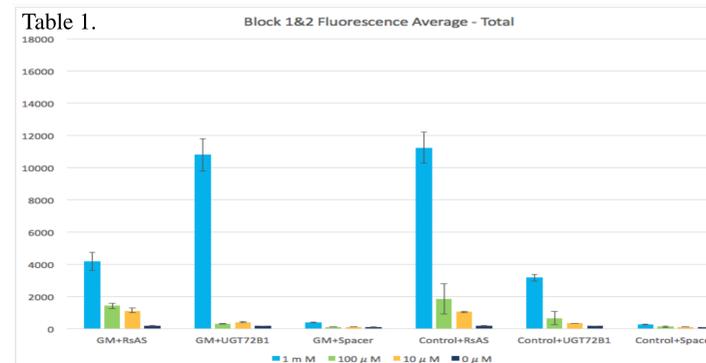


Table 1. Esculin Data:

The Green Monster and control produced similar amounts of fluorescence. In most cases, the production of Esculin was consistent between the experimental and control strain.

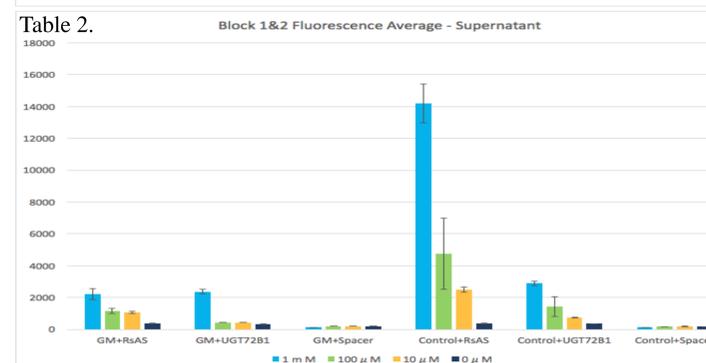


Table 2. Esculin Data:

The Green Monster strain produced significantly less fluorescence in the supernatant compared to the control. The Green Monster cells retained most of the Esculin produced inside the cell.

References:

- [1] Aesculin [Digital image]. (n.d.). Retrieved July 29, 2017, from <https://en.wikipedia.org/wiki/Aesculin>
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- [3] Dueber, J., Modavi, C., Grewal, P., & Russ, Z. (n.d.). Bioproduction of a betalain color palette in *Saccharomyces cerevisiae*. *Submitted*. Retrieved July 30, 2017.
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Results

Betanin Results

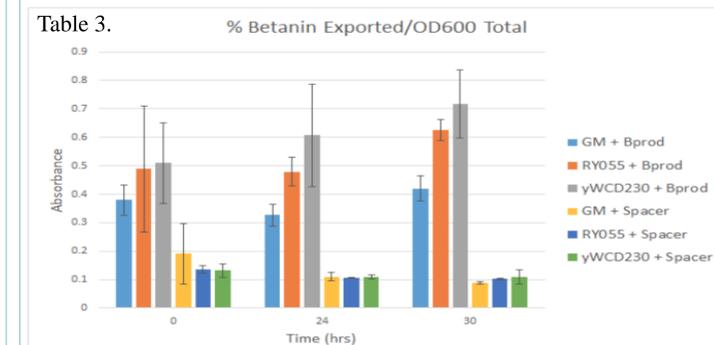


Table 3. Betanin Data:

Green Monster, its control, and a general yeast strain were tested in this experiment. The Green Monster produced less red color in the supernatant than the other strains.

Conclusions

The Esculin and Betanin pathway experiments both showed that the glucoside export was inhibited in the Green Monster strain. This revealed that one or more of the 16 ABC transporters removed in the Green Monster strain is involved in the exportation of glucosides. These experiments narrowed down the possible transporters to only 16 transporters.

Future experiments can begin to individually explore each of the 16 ABC transporters and their ability to export glucosides. Individual addbacks and knockouts can be performed for each transporter gene to test for the affect on the amount of coloration and fluorescence outside of the cell.

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