BioBuilderClub

Biosensor: Amatoxin-Containing Mushrooms

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Introduction

The *Amanita phalloides,* also known as the Death Cap, is one of the most poisonous mushrooms in the world. Half a cap of this mushroom, about 30 grams, is the estimated amount of a lethal dose to a human. One of the toxins responsible for its high toxicity is alpha-amanita. This is due to the toxin inhibiting RNA polymerase II, which negates production of mRNA, leading to cell death.

It has often been mistaken for other edible mushrooms, as shown below.

Fig.1 Deadly *Amanita phalloides,* Death Cap Mushroom

Fig. 2 Edible *Amanita calyptroderma,* Coccora Mushroom

Design

Our objective is to create a biosensor using a cell-free extract of *Saccharomyces cerevisae* yeast, plus a GFP transformed yeast plasmid to produce a green fluorescence when amatoxin is not present. In the presence of amatoxin, the extract does not produce fluorescence because the toxin inhibits RNA polymerase II, preventing protein production. The control is the cell-free extract containing the GFP yeast plasmid and water, which produces green fluorescence.

Methods

To test for the deadly amatoxin in an *Amanita* mushroom, our biosensor utilizes a yeast plasmid, transformed with a superfolder Green Fluorescent Protein (GFP) gene, and a freeze-dried cell-free yeast extract.

Prior to the test, the plasmid is added to the water which rehydrates the freeze-dried extract. The promoter of the GFP plasmid will be activated, and GFP production will begin. To eliminate false positives from carryover GFP, the mushroom must be added at the same time as the water and plasmid.

If the mushroom added does not contain the amatoxin, then GFP will be produced, telling the collector that the mushroom is edible.

Amatoxin will inhibit production of GFP, resulting in no green fluorescence. This will tell the collector it is not edible.

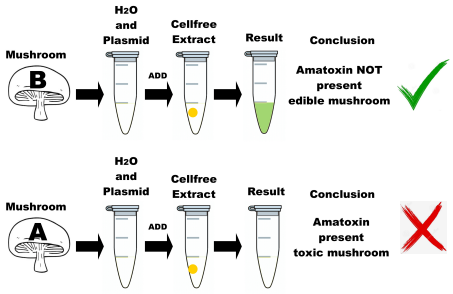

Fig. 3 Schematic of Test Procedure
Test mushroom + Water and Plasmid + Cell-Free Extract
Produces GFP if amatoxin is **NOT Present**
Or Does Not Produce GFP if amatoxin is **Present**

Materials

- 4 x 1.5 ml mini-centrifuge tubes
- MiniPCR P51 viewer
- *Saccharomyces cerevisiae* yeast, cell-free extract
- 0.01 g scale

Fig 4. Field Test Kit: 4 x 1.5 ml mini-centrifuge tubes, pipette, 0.01g pocket scale, P51 viewer, gloves, sample collection toothpick.

Preparation

- Prepare freeze-dried, cell-free, yeast extract
- Transform yeast plasmid with GFP gene
- Add plasmid to 0.5 ml of dH_20

Procedure

1. Obtain a 1 gram sample of the test mushroom
2. Crush sample in 1.5 ml tube, marked "test"
3. Mark a second tube as "control"
4. Add 0.5 ml of dH_20 (plus the transformed yeast plasmid) to both tubes
5. Add yeast extract to both tubes
6. Monitor production of GFP using P51 viewer for 30 minutes.

GFP Yeast Plasmid

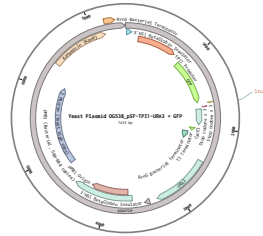

Fig. 5 Superfolder GFP Yeast Plasmid

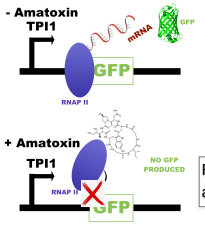

Fig. 6 Diagram showing that amatoxin inhibits RNAPII —> No GFP

References & Acknowledgements

Thank you to our mentor Dr. Jason Boock, Miami University, for all the guidance and help with our project and to Dr. Heather Hallen-Adams, University of Nebraska-Lincoln, for providing generous technical advice on the *Amanita phalloides* mushroom. Thank you to our teacher Ms. Barsever for the continuous support and advice on this project, the moral support and help of Melika Rezai and Katherine Fong and to Alverno Heights Academy.

Snapgene Superfolder GFP sequence
Oxgene pSF-TPI1-URA3 (OG538) Strong Promoter Yeast Plasmid
Yeast Cell-free extract: Cheng Wu, Matthew S. Sachs, Texas A&M University. Preparation of a *Saccharomyces cerevisiae* Cell-Free Extract for In Vitro Translation