Swanson-Holcomb Undergraduate Research (SHUR) Grant Proposal

Impact of ploidy on antifungal resistance

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Summary:

In order for evolution to occur, mutations in the DNA sequence are essential, which allows for new beneficial functions for the mutated organism. Some fungal species have mutations in their genome that allow them to become resistant to antifungal drugs, and it is thought that higher rates of mutations may drive higher rates of antifungal drug resistance[1]. Mutations in various genes impacting the function of DNA repair pathways (mutator alleles) contained in the genome of fungi can enhance antifungal drug resistance, allowing fungi to prevail despite the efforts to prevent their growth. Haploid (cells containing one copy of their chromosomes) and diploid (cells containing two copies of their chromosomes) cells develop resistance in different ways. Our prediction for this project is that mutator alleles promote drug resistance in haploids but not diploids. The goal of this project is to compare the rates of resistance between haploid and diploid cells, with and without the loss of function of a gene involved in DNA repair to determine the effect ploidy (number of copies of each chromosome a cell contains) has on the rate of antifungal resistance.

Introduction:

Despite the current treatments for invasive fungal infections, around 1.5 million people die from these infections every year, which is three times more than the number of annual deaths from malaria[2]. Antifungal drugs to treat these infections are becoming more ineffective as the fungal strains become more resistant to these antifungal drugs. Antifungal resistance refers to the ability of fungi to grow despite being in levels of an antifungal drug that is known to prevent fungal growth[3]. Resistance arises from mutations in the genome of an organism. Often, mutations are detrimental to an organism, leading to death and/or the inability to reproduce; however, sometimes, these mutations can be beneficial to the organism, such as allowing them to become resistant to a drug targeting them or allowing a protein to function more efficiently. To survive an ever-changing environment, an organism must find a balance between mutating enough to allow for these beneficial outcomes, but not enough to result in death[1]. Bacteria have

been known to mutate at a higher rate, as seen in antibiotic resistance rates. For bacteria, this higher mutation rate is largely facilitated by outside stress in the environment. In recent studies, it has been suggested that infectious fungal strains may also develop mutations in response to stress, whether that be from an antifungal drug or a challenge within its host[1]. Overall, fungal infections are becoming more and more difficult to treat, as antifungal drugs become ineffective. To model antifungal resistance, we will use *Saccharomyces cerevisiae*. This allows for the study of one criterion (such as ploidy), and its effect on resistance. It has been shown that haploid cells can develop resistance due to the presence of mutator alleles, but the effect of mutator alleles in diploid cells remains unknown. Mutator alleles are genes that have an error in the DNA such that DNA repair pathways are impacted and are not able to function properly. One such mutator allele is MLH1. The MLH1 gene encodes for a protein that has a key role in fixing errors in DNA during replication[4].

The main goal of this project is to determine the effect that ploidy has on antifungal resistance by comparing the haploid and diploid strains, with and without a deletion of the MLH1 mutator allele, of *S. cerevisiae*. This will allow for a better understanding on how antifungal resistance arises and how antifungal resistance may be able to be prevented.

Materials and Methods:

Saccharomyces cerevisiae models

S. cerevisiae is a widely used model organism for eukaryotic organisms. *S. cerevisiae* is a simplified eukaryotic cell that provides insight and the framework for what may be occurring in other eukaryotic cells during fungal resistance. Haploid strains are already available, and we can mate both of the haploid strains and select for the diploid strains.

Polymerase Chain Reaction (PCR)

In order to determine the identity of the *S. cerevisiae* constructs that will be used, PCR is needed. This allows us to see if the expected cut sites and deletions are present.

Minimum Inhibitory Concentration (MIC) Assay

After the identity of the *S. cerevisiae* strains is confirmed, a MIC assay shows how resistant a fungal strain is to the antifungal drug that is being used in an attempt to prevent its growth. The strains are added to a 96-well plate, and a range

of concentrations of fluconazole is used. The lowest concentration of fluconazole that prevents the growth of *S. cerevisiae* is the MIC value[5].

Resistance to fluconazole

Plate colonies that have been grown in fluconazole and count the number of colonies that grow, despite being grown in an antifungal drug.

Timeline:

Description	**Expected Time**
Mate haploid strains to make diploid strains. Grow strains of *S. cerevisiae* (control haploid, control diploid, ΔMLH1 haploid, and ΔMLH1 diploid) by streaking them on plates with media and incubating.	September-October
Design primers to run PCR to check the identity of the *S. cerevisiae* strains.	September-October
PCR the four *S. cerevisiae* strains to confirm that their expected identity and loss of function (for ΔMLH1 haploid, and ΔMLH1 diploid) is as expected.	October
MIC assay to determine antifungal resistance	October-November
Determine the strains' drug resistance to fluconazole by plating the strains after they have been exposed to fluconazole and counting the number colonies that grow. Results will be compared among the four strains.	October-December

Repeat process with new deletions and/or a new antifungal drug	January and beyond

Spring Research Symposium presentation	May

References:

1. Billmyre, R.B., Clancey, S.A., Heitman, J. (2017) Natural mismatch repair mutations mediate phenotypic diversity and drug resistance in *Cryptococcus deuterogattii*. *eLife* **6**:e28802 2. Firacative, C. (2020) Invasive fungal disease in humans: are we aware of the real impact? *Mem Inst Oswaldo Cruz* **115**:e200430

3. Berman, J., Damian, J.K. (2020) Drug resistance and tolerance in fungi. *Nature Reviews: Microbiology* **18**, 319-331.

4. MLH1 gene (2020). MedlinePlus [Internet]. Bethesda (MD): National Library of Medicine. [2022 September 18]. Available from: https://medlineplus.gov/genetics/gene/mlh1/ 5. Rodríguex-Tudela, J.L, Barchiesi, F., Bille, J., Chryssanthou, E., Cuenca-Estrella, M., Denning, D., Donnelly, J.P., Dupont, B., Fegeler, W., Moore, C., Richardson, M., Verweij, P.E. (2002) Method for the determination of minimum inhibitory concentration (MIC) by broth dilution of fermentative yeasts. *Clinical Microbiology and Infection* **9**, 1- 8.

Budget:

Item	Description	Cost
96-well plates (from VWR)	Used for *in vitro* evolution experiments and MIC assays to determine resistance of *S. cerevisiae* to fluconazole.	~$300
Fluconazole (from GoldBio)	Antifungal drug used for the selection of drug resistance mutants.	$121

PCR primers (from IDTDNA)	Used to confirm the strains of *S. cerevisiae* with and without deletions of mutator genes. This is an essential part of the project in order to determine if the strains are indeed the ones that we want to work with.	$80
		Total Cost: $501