Mutagenesis of *Saccharomyces Cerevisiae*:

Producing and characterizing mutants of the adenosine biosynthesis pathway

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Abstract

The purpose of this experiment was to create and identify mutants of the adenosine biosynthesis pathway in the yeast species *Saccharomyces Cerevisiae,* and to determine whether the mutations are affecting the ADE1 or ADE2 gene. When the genes ADE1 or ADE2 are disrupted, the cells can take on a reddish/pinkish hue due to the buildup of a red pigment. The pathway converts AIR to CAIR using the ade2 enzyme, which is then converted to SAICAR using ade1 enzyme. Disruption of this causes the cell to need an exogenous source of adenine. The Experiment was started out by obtaining an LD50 and LD80 from a survival curve created with class data. The LD50 was 8 seconds, and LD80 was 18 seconds. These times were used to create mutant cells in hopes of producing mutants in the adenine pathway, however this did not work so we needed to use mutant cells produced by another group. A complementation test was later used on wild type colonies that appeared in a plate grown from mutant cells, this was done to determine if the reversion to the wildtype phenotype was due to the interaction of mutants on different genes. Complementation occurred crossing HA1 with HB2, XB4, and XB6. Complementation also occurred crossing HA2 with HB1 and XB5. This suggests that the mutations present in HB2, XB4, and XB6 strains are in the ADE2 gene, and the mutations present in HB1 and XB5 are in the ADE1 gene.

Introduction

The species of yeast *Saccharomyces Cerevisiae* is commonly used to cook bread and brew beer; however, it has been widely utilized by the scientific community as a model eukaryotic organism in genetic research. Using this species of yeast allows one to produce many generations containing many cells in an incredibly short amount of time. This property of yeast as well as how little it costs to obtain and main is the reason why we selected *Saccharomyces Cerevisiae* for this experiment.

Adenosine is a nucleoside that is involved in many important biological processes such as the storage of energy in the form of Adenosine triphosphate, as well as an inhibitory neurotransmitter in the CNS in mammals [1](Sperlagh & Vizi, 2011). Adenosine is composed of adenine and d-ribose, each of which playing important biological roles in genomics and energy storage. The adenosine biosynthesis

[1] Sperlágh, B., & Vizi, E. S. (2011). The role of extracellular adenosine in chemical neurotransmission in the hippocampus and Basal Ganglia: pharmacological and clinical aspects. *Current topics in medicinal chemistry*, *11*(8), 1034-46.

pathway is the multistep enzymatic pathway in which the cell produces adenosine to be used for various biological processes. There are two steps within this pathway controlled by the ADE1 and ADE2 genes that help the cell produce its own adenine to be used for later steps in this pathway. Normally in this pathway AIR is converted to CAIR by ade2, and then CAIR is converted to SAICAR by ade1. When this is disrupted due to mutation, the cell will need an exogenous source of adenine to survive.

The phenotypic effect of mutation to the ADE1 and ADE2 genes leads to yeast cells taking on a slightly reddish hue, opposed to the creamy white color found in the wildtype[2] (Sharma, Kaur & Bachhawat, 2003). Due to the distinct phenotype this mutation generates, we will be able to identify the desired mutants from the wildtype.

The first part of this project was exposing varying concentrations of yeast cells to UV light for varying durations to find and LD50 and LD80 giving us a sense of how long to expose our cells to in order to create the mutants. Once the mutants were created, we needed to test to see if the mutants were either ADE1 or ADE2, as well as see if any other mutations were present in the pathway. The ADE1/ADE 2 mutants were recovered by doing a UV mutagenesis screen, which was followed by a complementation test in order to determine if the mutants were in the ade1 or ade2 genes.

The mutagen we chose to cause mutations in the ADE1 or ADE2 genes was ultraviolet radiation around the 254nm range. UV radiation causes dimerization of pyrimidines [3](Rastogi, Richa, Kumar, Tyagi & Sinha, 2010) (cytosine or thymine) in a cell's DNA which can lead to a gene losing its function if it is not

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[2] . Sharma, K. G., Kaur, R., & Bachhawat, A. K. (2003). The glutathione-mediated detoxification pathway in yeast: An analysis using the red pigment that accumulates in certain adenine biosynthetic mutants of yeasts reveals the involvement of novel genes. *Archives of Microbiology*, *180*(2), 108-117. doi:10.1007/s00203-003-0566-z

[3] Rastogi, R. P., Richa, Kumar, A., Tyagi, M. B., & Sinha, R. P. (2010). Molecular mechanisms of ultraviolet radiation-induced DNA damage and repair. *Journal of nucleic acids*, *2010*, 592980. doi:10.4061/2010/592980

repaired. 254nm is an optimal wavelength for causing the desired mutation, as longer wavelengths are too low energy and would take a significant amount of time, but lower wavelengths would be too high energy and cause excessive mutation.

Methods and materials

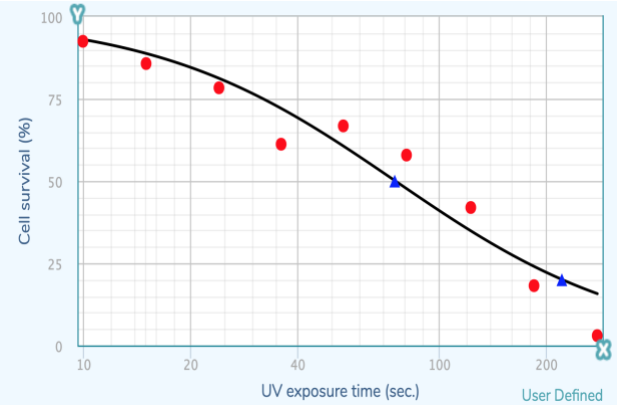
Three YED plates were labeled based on the cell dilution and radiation time, and then coated with 100 microliters of the associated cell solution using glass stirring beads. Each plate was exposed to its designated amount of UV radiation, and then placed upside down in the 30°C incubator for 3 days. After the three days have elapsed, the plates were placed in the 4°C incubator until the next lab section. The colonies were counted using a sharpie to mark the plate, and the data was entered into a class data chart to create a survival curve, allowing us to determine the LD50 and LD80 of radiation exposure. Red/pink colonies were saved for the next lab section and the data were recorded in our lab notebooks. In the next lab section, 100 microliters of HBO 10^3 cells/ml were placed on plate #1 to be used as a control (no UV exposure.). 100 microliters of 10^4 cells/mL were spread on LD50 and LD80 plate. LD50 and LD80 plates were exposed and then all three plates were placed in the 30°C incubator for 3 days and then 4°C incubator till the next week. The plates were collected, and WT and red colonies counted separately. These data were used to calculate a mutation rate and a survival rate. Mutant colonies were counted and streaked onto a new plate. This plate was then incubated at 4°C until next the week. After incubation, three mutants were selected and plated. Once plated, mutants were crossed by streaking the yeast cells corresponding to each column in the crossing grid. Once all crosses were performed, the plate was incubated for three nights at 30°C and the rest of the week at 4°C. A picture was taken of the cross plate, and the genotypes were

determined for each of the unknown strains tested, as well as the unknown mutants. This was done to see if complementation had occurred between any of the yeast strains.

Results

The LD50 and LD80 were obtained by generating a survival curve (figure A and C) using class data, and then calculating the predicted percentage of cell survival for 50% and 20% using the equation “y = (100)/(1+(x/c)^b)”. The LD50 and LD80 as shown in Figure B. are 75.2196 seconds and 221.7509 seconds respectively.

1.Figure A. Survival curve



2. Figure B. LD50 and LD80

Cell survival (%)	UV exposure time (sec.)
50	75.2196
20	221.7509

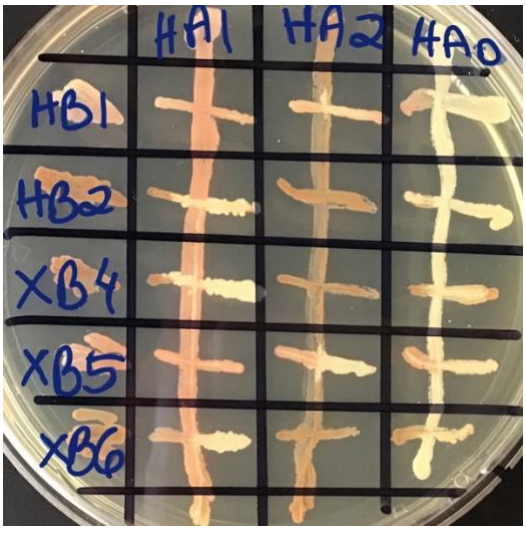
3. Figure C. Survival curve dasta.

UV exposure time (sec.)	Cell survival (%)

0	100
10	92.5
15	85.7
24	78.3
36	61.2
54	66.8
81	57.9
123	42.0
185	18.2
278	3.01

Our mutagenesis failed so we had to use stock plates. Our complementation (Figure D) test showed that complementation occurred between HA1 and HB2, XB4, XB6, as well as HA2 with HB1, XB5.

4. Figure D. Complementation test



Discussion

Through creating a survival curve with all the class data, we were able to find the LD50 and LD80 for the cells in order to determine the optimal exposure for mutagenesis. The LD50 was exposure for 8 seconds, and the LD80 was exposure for 18 seconds. We used this information to undergo mutagenesis in our experimental; plates. We were unsuccessful in producing mutants of our own, so we had to use mutant colonies obtained by another group.

The complementation test showed us if any mutations complement the original mutation, meaning the mutation occurred on an allele of a separate gene, restoring the wildtype phenotype. We conducted the complementation test by streaking known mutants with unknown mutants on a plate in grid formation, the intersecting streaks will show both mutant red phenotype as well as the white wildtype phenotype depending on whether the mutations complement each other or not. What we found was that complementation occurred at the crosses between HA1 with HB2, HB4, and XB6. Complementation also occurred at the crosses between HA2 with HB1 and XB5. HAO was wildtype and the phenotype showed up in every cross. HA1 and HB1 were mutants for the ADE1 gene and HA2 and HB2 were mutants for the ADE2 gene. If complementation occurs at the cross between a given mutant and HA1 but not HA2, this means that the mutation is within the ADE2 gene. The reversal of this is also true, meaning if complementation occurs between a given mutant and HA2 but not HA1, this means the mutation is within the ADE1 gene. The Reversion back to the wildtype phenotype could occur through complementation, or less likely through total reversion due to a reversal of the mutation that caused the red phenotype.

Reference:

Rastogi, R. P., Richa, Kumar, A., Tyagi, M. B., & Sinha, R. P. (2010). Molecular mechanisms of ultraviolet radiation-induced DNA damage and repair. *Journal of nucleic acids*, *2010*, 592980. doi:10.4061/2010/592980

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