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Benzo(a)pyrene Induced Mutagenesis of *Saccharomyces cerevisiae*

Hannah Fisher

ABSTRACT

Saccharomyces cerevisiae is a species that is ideal for research. It is easy to obtain, grow and manipulate. The relatively fast life cycle makes it the optimal choice for inducing mutations. Benzo(a)pyrene is a polycyclic aromatic hydrocarbon. It has been labeled a cancer causing agent. The effects depend on dose, duration, pathway, other chemical exposure and individual characteristics. BaP is of special concern because contact with it is virtually unavoidable. It has been found in almost all environmental media and food ingested by humans and animals. A mutation that results from the exposure of a cell to exogenous DNA modifiers such as chemicals or radiation is termed an induced mutation. The objective for this experiment was to induce and observe mutations within the yeast genome. The yeast was grown and BaP was added to the experimental group. The diluted broths were plated on a plate containing a nutrient broth. After an incubation period, DNA was extracted from the colonies. The DNA was cut with restriction enzymes and the amplified with PCR. Then electrophoresis was run. Due to problem in the electrophoresis procedure, there were no results. The anticipated results were that there would be changes in the size of the DNA fragments in the gel. Had there been results, the rate of mutagenesis could have been found. Future studies should allow for more time to make sure that each step is successful.

Keywords: *Benzo(a)pyrene, Saccharomyces cerevisiae, Polymerase chain reaction, electrophoresis, induced mutation, restriction enzymes*

INTRODUCTION

Saccharomyces cerevisiae (yeast) has been a model organism for research for over 50 years (Curran, 2006). There are many aspects of *S. cerevisiae* that make it ideal for research. This fungus is a unicellular eukaryotic species that is genetically tractable (Atlas, 1997; Winzeler, 1999). *S. cerevisiae* colonies grow rapidly on a simple medium with cells doubling approximately every 100 minutes (Herskowitz, 1988). These factors contributed to the choice of *S. cerevisiae* for my experiment. Yeast is readily available, inexpensive and there are research references available for comparison (Sherman, 2000).

Yeast will grow and reproduce on a minimal medium containing appropriate sources of carbon, nitrogen, basic minerals, vitamins and salts. Cells grow most rapidly at 37C (Winzeler, 1999). *S. cerevisiae* is a budding yeast in which the “mother” cell produces a daughter cell made of new surface material (Atlas, 1997; Herskowitz). *S. cerevisiae* has a life cycle that consists of both haploid and diploid stages (Winzeler, 1999).

Polycyclic aromatic hydrocarbons (PAHs) are a combination of chemicals that occur naturally during the incomplete burning of organic substances (ATSDR, 1995). The effect of PAHs as cancer causing agents depends on dose, duration, pathway, other chemical exposure, and individual characteristics (Atlas, 1997; ATSDR, 1995). Benzo(a)pyrene (BaP) is one of the many PAHs that have been identified as a probable carcinogen by the EPA (EPA, 2005). Benzo(a)pyrene is of special concern because it has been found in almost all environmental media and food items ingested by humans and animals (Hattemer, 1991). BaP has been found to cause tumors in lab animals, and with long term contact or inhalation causes cancer in humans (IARC, 1983). BaP is a solid, pale yellow compound with a faint aromatic odor and has a needle-like crystal structure (ATSDR, 1995). The five ring structure is recalcitrant. The 2005 CERCLA Priority List of Hazardous Substances list BaP as number nine (ATDSR, 2005).

An induced mutation is defined as a mutation that results from the exposure of a cell to exogenous DNA modifiers such as radiation or chemical substances (Atlas, 1997). Mutations can be identified by changes in DNA or changes in the observed phenotype. DNA mutations can be in the form of additions, deletions or substitutions of the nucleotides (Atlas, 1997). In my experiment I will be trying to induce mutation in *Saccharomyces cerevisiae* by exposing it to BaP. To identify any mutations I will be looking for changes in the DNA sequence by observing differences in the sizes of DNA fragments created by restriction endonucleases.

MATERIALS AND METHODS

Saccharomyces cerevisiae wild type HAO strain was purchased from Carolina Biological Supply. Two test tubes, each containing 10mL of nutrient broth, were inoculated with the yeast and were incubated at 37C for 48 hours. The incubation time is based on the fact

that *S. cerevisiae* doubles in number every 100 minutes (Herskowitz, 1988). Therefore in the 48 hours the *S. cerevisiae* will have doubled in number up to 28.8 times. The tubes will be incubated at 37C because it is the optimal growth temperature of *S. cerevisiae* (Xiao, 2006).

After the incubation period, 2mL of diluted DMSO was added to one test tube, which served to be the control group. An allotment of 2mL of 0.03% BaP and DMSO mixture was added to the other test tube, which comprised the experimental group. To achieve the desired 0.03% concentration the BaP was dissolved in dimethyl sulfoxide (DMSO) and then added to deionized water. The DMSO must be used because BaP has low water solubility (Hattemer-Frey, 1991). The two tubes were then incubated for 2 hours at 37C. This time lapse was to allow the yeast to reproduce but not long enough for the mutations to kill the *S. cerevisiae* cells. The absorbency was then read using a spectrophotometer to determine the yeast cell population. The yeast broth was then diluted by a factor of 10^-6to 10^-10 to reduce the cell population to a countable number. The cell population was diluted to between 100 and 500 cells per mL. This was done by taking 0.1mL and transferring it to 10mL of nutrient broth. This was repeated 4 more times and the last three tubes of diluted broths were put onto plates.

An agar recommended by Carolina Biological Supply, MV media was used to make the plates. An aliquot of 1 mL of diluted broth culture was pipetted onto each plate. The plates were then incubated for 48 hours. Colonies were then isolated for DNA extraction.

DNA was extracted from all of the cultures using the Epicentre MasterPure Yeast DNA Purification Kit purchased from Epicentre. The procedure from the kit was followed. A TBE buffer purchased from Carolina Biological supply was added to the DNA. The DNA-buffer solution was then amplified using Polymerase Chain Reaction (PCR). To accomplish the amplification an Epicentre FailSafe PCR System containing all of the reagents needed was purchased from Epicentre and its procedure was followed. The PCR technique was used because of the ability to amplify small DNA fragments.

The amplified DNA was then cut with a combination of Hind III, and EcoR I restriction enzymes purchased from New England BioLabs. The control yeast DNA and experimental DNA were cut the same to allow for comparison.

Agarose gels (0.08%) were made to run electrophoresis to allow for visibility of DNA fragment banding patterns. To make the agarose gel, the powered form was mixed with deionized water in a flask. The water was then heated, causing the agarose to dissolve. While the mixture was being heated, the flask was swirled approximately every 20 seconds. When the correct consistency was reached, a thermometer was added to the solution. The mixture was allowed to reach 60C to prevent warping of the tray. The gel was then poured into casting trays containing a comb to make wells. After sitting for 20 minutes, 20 micrograms of DNA was inserted into wells (Wiley, 1988). Lambda DNA was inserted in each of the end lanes to allow for easier comparison.

Electrophoresis was run at 80 volts for 105 minutes. After electrophoresis was ran, a Carolina Blu Stain purchased from Carolina Biological Supply was used to make DNA bands visible. The DNA banding patterns were then examined to determine if there were any mutations between the control sample and the sample exposed to BaP.

RESULTS

The anticipated result for this experiment was that there would be a difference in the appearance and sizes of the DNA banding. Due to problems during electrophoresis no DNA banding was visible. One of the gels was flooded during addition of the salt solution to the chamber. The other gel was run with the tape left on at first. Once the problem was corrected, the gel was already dried out and DNA was unable to migrate.

DISCUSSION

As a result of no gel being produced after electrophoresis, it is unknown whether the previous steps of the experiment were performed properly. Had there been detectable mutations in the experimental DNA, the rate of BaP mutagenesis could have been determined. Similar banding patterns in the control and experimental group would not have meant that there was no mutation. It would have only indicated that there were no mutations at the sites the restrictions enzymes chosen had cut. The experiment could have been repeated with various other restriction enzymes. Future studies should allow for more time to adequately check at each step to insure that the experiment is progressing properly.

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