Original Article

# Brewer's yeast resistance to X-ray radiation

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## Abstract

Various species of bacteria have adapted to man-made chemicals and antibiotics, developing into superbugs posing a threat to humankind, and soon creating a future where antibiotics are futile against various diseases. In recent studies, the gold nanoparticle were tested in photothermal therapy to treat cancerous cells, as gold has conductive properties that make it an excellent medium for electromagnetic dissipation. The silica and polyethylene glycol coating was selected, because its properties that prevents the deformation of gold nanoparticle when exposed to NIR laser at 808 nm with 500mW and green laser 520nm with 100mW. The angle of divergence and radius of light at any given distance equations were incorporated to finding the time and distance for the laser set up. The resonating gold nanoparticles irradiated by the infrared laser produced heat radiation that eradicated the E. coli strain k12 and Staphylococcus Epidermidis within 24mm and 26mm zones of inhibition, respectively. This compared favorably to penicillin as it created a 27mm zone of inhibition. The determination of the heat that the particles produced was calculated with the equation describing the heat of a system that is energized by electromagnetic radiation. The photothermal therapy method demonstrated a potential to treat bacterial infection.

**Keywords:** Brewer's yeast resistance to X-ray radiation.

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## INTRODUCTION

**Purpose**

The objective of this experiment is to test the effect of x-ray radiation on micro organismal development. We will be looking at the changes in the structure and growth of brewer's yeast after exposure periods to low voltage x-ray radiation. The purpose of this experiment is to demonstrate the effect of the frequency of radiation dosages on the regenerative capability of brewer's yeast.

**Variables:**

## INDEPENDENT VARIABLE

The independent variable is the interval of time between each dose of radiation. Group C (1-4) will be exposed to 20 kilovolts of radiation a total of five times over the course of five days, Group D (1-4) will be exposed to 20 kilovolts of radiation every other day (five times over the course of ten days), and Group E (1-4) will be exposed to 20 kilovolts of radiation every third day (five times over the course of 15 days).

**Dependent variable**

The dependent variable is the rate of growth or decline of the yeast and other quantitative changes that occurs. In addition, qualitative measurements will be taken continuously of the brewer's yeast shape, form, and structure.

**Constants**

1. Seconds of radiation exposure
2. Type of x-ray tube
3. The setting
4. No light
5. Room temperature
6. Culture incubated at 30° C
7. Amount of yeasts in each petri dish
8. Type of brewers' yeast
9. Total dosage of radiation
10. Amount of nutrient materials in the empty petri dish

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## BACKGROUND INFORMATION

Brewer's Yeast is formed from a single celled fungus known as ***Saccharomycescerevisiae (umm.edu).*** Today, Brewer's Yeast remains to be one of the most commonly used experimentation subjects due to their rapid growth periods and easy accessibility (nytimes.com). Their genetic structure has made them one of the most crucial components in modern evolutionary research. Grown in an insulated dish with adequate water and sucrose, they rapidly develop into large defined colonies, making them a capable indicator of structural changes as a result of external stimuli (bio.davidson.edu). Their reproductive process is known as budding in which the parent organism develops an external growth that further branches off into a new organism. Yeast has been critical to the development of human biology as well. It is through these cells that we have developed a functional understanding of the cells which control the cell cycle, cell apoptosis, signaling proteins, and protein processing enzymes (Walker, 11). The mass of a single yeast cell is approximately $2.4*10^{-11}$ grams with an uncertainty of 16.9% (ncbi.gov). The mass of the petri dishes is 25 grams and the mass of the sugar solution is 13 grams, leaving two grams for the mass of the yeast. Factored in with the mass of a single gram, there are approximately 83,000,000,000 cells of yeast in the solution. The key components of the x-ray machine include the x-ray tube, the power supply, and the radiographic table. The most common scientific unit measurement of x-ray radiation dose is the millisievert (mSv) and other radiation dose measurement units include rad, rem, roentgen, sievert, and gray. The x-ray produces radiation from energy taken from electron and converts it into photons. The process of radiation production takes place in the x-ray tube. The x-ray tube's primarily function is to convert energy; it receives the electrical energy in the form of kilovolts from the high-voltage power supply, then the cathode focuses the electrons into a fine line and converts the energy into radiation at the anode which directs the energy flow. The electron flows into the x-ray tube in the electrical circuit. As the electrons flows into the x-ray, they carry an electrical potential energy with them. Then the electrical potential energy is converted into kinetic energy or motion, which is finally converted to radiation and heat. (http://www.sprawls.org/). The x-ray tube contains a cathode and anode. The generation of x-ray radiation occurs, as the electrical flows through the tube from cathode and anode. The anode is usually the large piece of metal that connects to the positive sides in the electrical circuits and it is where the radiation is produced. The cathode usually consists of a small coil wire called the filament, which is recessed in a cup shaped region and is negatively charged. Through thermionic emission that occurs in the cathode, thermal energy (heat) excites and releases the unbound electrons.(http://www.sprawls.org). The generation of ionizing radiation has significant adverse effects on the DNA of yeast cells. When ionizing radiation (radiation that results in the loss of an electron) strikes DNA, it results in a double breakage across the length of the DNA. Enzymatic repair mechanisms are often effective at repairing this damage, but they occasionally encounter errors in which wrong portions of the DNA is reattached to the wrong portion creating a mutation. Mutations can be silent, influencing the third base pair of a codon and resulting in largely no changes, or frameshift mutations, resulting in the failure of the cell. In some cases, genetic abnormalities result in apoptosis, preventing further damage to a multicellular organism. This is not always the case, however. Damaged DNA can shut down apoptotic pathways resulting in cancerous cell growth.

## CONTROL/EXPERIMENTAL GROUPS

The two Control Groups are A and B, each containing two petri dishes. The Control Groups A and B will not be exposed to any radiation and they will contain yeast contained in a petri dish for the duration of the experiment. The Experimental Group C1, C2, C3 and C4 will contain yeast that will be exposed 20 kilovolts of X-ray radiation for 10 second 5 times over 5 Days. The Experimental Group D1, D2, D3 and D4 will contain active yeast that will be exposed to 20 kilovolts of radiation for 10 seconds, 5 times over 10 days. Experimental Group E1, E2, E3, and E4 will be exposed to 20 kilovolts of radiation for 10 seconds every third day for 15 days.

**Hypotheses/Goal**

If 3 groups of yeast are exposed to the same level of radiation at varying intervals, then the group exposed to radiation doses every third day will demonstrate higher relative growth and less structural damage while group as repair mechanisms can prevent abnormal activity in a cell if given adequate recovery periods while the group exposed to radiation daily will experience a sharp increase in growth followed by a rapid period of cell death due to mutations caused by breakages in DNA. The goal of this experiment is to determine the ability of cells to recover from radiation exposure.

**X-ray Machine Materials**

- X-ray Tube
- Power supply capable of supplying up to 25 kilovolts of electricity
- Black 12 AWG hook up wire 20 feet
- Red 12 AWG hook up wire 20 feet
- Five alligator clips
- 441 square inches of lead radiation shielding minimum of 1/32 inches thick

- Electrical tape
- Dosimeter
- 1" screws
- 4 10X9 wooden planks
- 1 9X9 wooden plank
- Electric drill
- Hammers

**Yeast Materials**

- Empty Petri Dishes (pack of 20)
- High fructose corn syrup
- Water
- Incubator
- Sterile tablespoon measuring tool
- Gloves
- Brewer's yeast
- Microscope

**Risk and Safety**

The X-ray was built in an electrically isolated system enclosed in lead to prevent any risk to those operating the experiment. The entire experiment was not just contained in a lead lined box, but the device was tested in an enclosed facility with a safety interlock to prevent accidental exposure to radiation. All construction of the equipment was inspected and tested by a designated supervisor to ensure that all standards of safety were met. The room had also featured a Dead-Man's switch and remote operation capabilities to ensure that participants did not face exposure to X-ray radiation themselves. The "Dead-Man's switch" was capable of automatically turning off all power sources in the room if radiation levels get too high in the external environment. The X-ray machine's lead line housing had no glass for visibility into the box.

**Steps followed to build the X-ray Machine**

1. The testing housing for this experiment essentially consisted of a small wooden box, reinforced to withstand X-ray radiations using sheets of lead measuring 1/16th of an inch in diameter. The box did not contain a base because the petri dishes were set up directly on top of a 1/8th inch lead shield that was used as a removable base.
2. Four 10X9 sheets of wood, each 1/2 inch thick, were laid in a cross pattern with a 9X9 wood plank in the center.
3. Holes were drilled in all four corners of two 10X9 wooden planks (the top and bottom of the cross) using an electric drill and then 1 inch screws were inserted in each of the holes using a hammer.
4. It was carefully ensured that the screws were inserted approximately one fingernail length away from the edge of the wood so that the wood doesn't split.
5. The four 10X9 planks in the cross were raised and the screws were hammered through the planks to make the structure solid.
6. The 9X9 planksthat constituted the lid of the box were added to the box. Four small holes will be drilled into the top and 1-inch screws were put through the top of the box into the supporting wooden planks.
7. The wood was then aligned with lead sheets on all 4 major sides of the inside of the box and on the top of the inside of the box. No inch of the wood was exposed on the inside, as the X-raysradiations could escape through a small gap in the shielding. The X-ray tube was mounted to the top of the inside cover of the box (over the led shielding) so that all radiation gets directed downwards (towards the location of the agar tray that did not constitute to be a part of the box. Since the trays were not part of the box, they were easily removable.
8. The box featured 2 small holes so that electric wires could be run from the high voltage power supply (stored outside the radiation compartment) to the X-ray tube.
9. The high volt power supply was placed on one side of the platform while the x-ray tube was mounted using an adhesive to the upper segment of the box.
10. All power was disconnected from the high voltage power supply and the supply remained OFF while the cables were being connected to the X-ray tube.
11. The alligator clips and 12 AWG were used to hook up a wire to connect the power supply to the X-ray tube.
12. It was crucial that the alligator clips attached the negative end of the X-ray tube and to the negative end of the power supply and the positive end of the X-ray tube to the positive end of the power supply.
13. (For the purposes of this experiment, a step down transformer was used to convert the 25 kv output of the high voltage power supply to a 20 kv output. This was attached to the wiring between the high voltage power supply and the X-ray tube.)
14. Once the x-ray tube was connected to the high voltage power supply, the shielding covered the x-ray tube.
15. While testing, it was made sure that a dociometer/KV meter was continuously used to

measure the μRem in the lab for safety. The dosimeter remained within the isolated compound in order to guarantee that X-ray radiation was actually being emitted from the device. The dosimeter will provide constant updates as to the levels of radiation and it will also ensure that the radiation levels in the surrounding area are not too high.

**Yeast Construction**

1. Count 20 sterile petri dishes
2. Add 10 g/liter of sucrose to the 1000 ml of distilled water and heat the distilled water using the magnetic stir plate until it boil
3. Add 21 grams/liter of dried nutrient agar
4. Wait until water comes to a boil again
5. Immediately distribute agar/sucrose/water mix into 20 petri dishes
6. Allow petri dishes time to cool at room temperature and allow agar to harden
7. Once the agar has hardened, combine 160 ml water with 10 grams of yeast to achieve a low viscous solution of yeast.
8. Use a sterile transfer tool to pleat the yeast over the agar, distributing yeast equally over the surface of the dishes
9. Allow 2 days for yeast growth prior to beginning the experiment

**Steps for experiment**

1. See instructions for construction of x-ray machine and yeast trays
2. Control Group A1-A4 and B1-B2 will not be exposed to any radiation and will be left in the enclosed containment for 15 days while the other fungi is tested. These containment units will be used to compare the growth and mass of the fungi.
3. Four prepared media plates will be labeled Experimental Group C1, C2, C3 and C4 on the exterior of the petri dishes, other four petri dishes will be labeled experimental Group D1, D2, D3 and D4 and the final 4 petri dishes will be labeled Group E1, E2, E3, and E4.
4. All the control group and experiment group will be filled with approximately .5 grams yeast using the plating technique.
5. All the yeast trays will be given one day as time for a growth period.
6. After one day, the yeast will be examined visually to ensure proper growth. The structure and quantity of the yeast in all the trays will be examined thoroughly prior to beginning and after the radiation testing.
7. The mass of the dishes with yeast will be measured using the scale.
8. Percentage yeast coverage will be computed by taking a picture and histogram program to measure approximate coverage of yeast cells everyday.
9. The x-ray emitter should be testelevels of radiation output.
10. Group C1-C4 will be exposed to 20 kilovolts of radiation ( 250 Counts Per Minutes, or.125 mR/h) for 10 seconds.
11. Step 10 will be repeated every day for five days. Record the data from qualitative observations from visual and microscopic evidence and the quantitative data of overall mass of the plates and percent coverage of yeast.
12. D1-D4 will begin testing on the same day as C1-C4. D1-D4 will be exposed to 20 kilovolts of radiation for 10 seconds immediately following the C group testing. Unlike the C group, however, which will be tested over the course of 5 days, the D group will be tested over the course of 10 days with radiation exposures every other day. Starting on the same day as the C group, the D group will be tested every other day. Record the data of the mass of yeast, the yeast percent coverage, the structure of the yeast from visual and microscopic evidence.
13. The same indicators will be measured for Group E. Group E, however, will be exposed to radiation every third day to determine regeneration capabilities.
14. Record the mass of the dishes, general qualitative observations of yeast cellular structure under a microscope, and coverage percentage of the trays.

## DATA ANALYSIS

In order to establish a measure of the growth rate of the yeast, two distinct measurement styles were used- changes in mass, and changes in the percentage of the petri dish that was covered with yeast cells. Mass was measured to an accuracy of 1/100th using a scale, while coverage was measured by calculating the number of pixels of yeast coverage divided by the total number of pixels of the petri dish in a picture of the tray. When graphing, averages of the four trays in each group were used as data points to avoid results being skewed by any particularity in a dish. Over the course of 15 days of testing, both control groups (A and B) demonstrated continuous, unimpaired growth. Mass testing revealed that decreases is masses signified growth, largely due to the consumption of nutrient agar and the relatively low mass of the yeast cells. For that reason, the greater the decrease in mass, the greater the growth. The mass of

both control groups decreased 4.26 grams from the beginning of the experiment and increased approximately 22% (combined) in terms of petri dish coverage. Statistical t-tests yielded a value of 0.336, indicating that the growth of the control groups were statistically identical and were therefore not the result of human error. It is for this reason that the control group can act as an adequate comparison for the test groups (C, D, and E). Group C demonstrated unique growth over the period of radiation exposure though its growth pattern eventually normalized to become comparable to the controls. In terms of mass, the C group had almost no change in the first five days of testing during which it was exposed daily, indicating negligible growth rates. 2 days after radiation exposures had stopped, however, the average mass of the C group began decreasing again at a rate consistent with the control groups, indicating that whatever genetic damage had prevented normal metabolic activities had been properly repaired. Similar results can be seen in the percentage yeast coverage analysis. The C group exhibited extremely low growth during the first five days of testing as coverage percentages stayed largely stagnant at 22%. 2 days after radiation testing had stopped, however, the growth rates returned to normal. In terms of qualitative measurements, it appeared that structurally, cells in the C group displayed less of a distinctive structure and a general lack of the colonies which were present in the control groups until radiation testing had stopped. Group D exhibited periods of elevated growth followed by decays in both coverage and nutrient consumption rates. During the first six days of testing in which group D was tested every other day, the mass of the petri dishes dropped dramatically, with the maximum decrease rate occurring during day 6. Similarly, the percentage yeast coverage of group D increased dramatically peaking at day 6 and then experiencing a decrease (the only group experiencing a decrease). The cellular structure of the yeast under a microscope had the distinctive colonies and independent cell structure of the control, yet these features largely disintegrated after the coverage began to decrease. This indicates that ionizing radiation exposures every other day may have resulted in mutations of the yeast cells which built up to the point where cells were forced to undergo apoptosis to prevent more damage. At the end of 15 days, overall growth rates compared with day 1 were at 17%, signifying some ability to correct the genetic damage. Group E was consistently similar to the control group. In mass measurements, coverage measurements, and qualitative data, the E group appeared identical to the controls. This indicates that radiation administered at intervals of 3 days have no significant adverse or positive effects on the growth rate of Brewer's Yeast cells.

## Evaluation

Experimentation on the ability of microorganisms to consolidate damage by ionizing radiation indicated that nucleases are unable to repair genetic damage except when they are provided adequate rest periods. Groups exposed to radiation daily experienced significant changes in structure and overall losses in mass while those groups exposed every third day experienced no significant losses in mass and demonstrated continued growth comparable with the control group. It is evident that photolyase and other enzymes responsible for correcting fungal genetic damage are incapable of repairing frequent genetic damage. Overall, it appeared that the hypothesis was partially correct, in that, Group E did demonstrate higher relative growth for the time as well as less structural damage. Group C, however, did not demonstrate increased growth initially, as the frequency of radiation dosages resulted directly in minimalized metabolic activity of the yeast cells. This may have largely been due to the fact that the mutations caused by daily genetic damage resulted in the inability of the cell to grow and develop as well as death rather than an exponential growth mutation (seen by Group D). Group D did display the greatest growth rate initially though it promptly experienced a period of decay. If this experiment were to be replicated, several changes could be made to ensure more distinctive results. If more test groups tested over longer intervals were added to the experiment observations of the ability of nucleases to correct genetic damage may have been more accurate. In addition, the inclusion of an incubator or other heat source for the yeast cells may have increased growth and allowed the researchers to more effectively view the impact of radiation intervals. In addition, using greater radiation levels may allow an investigation into the immediate effects of radiation on the cells in addition to long term damage.

**Application**

**Yeast as an Indicator in Human Spaceflight**

- Yeast has many of the same capabilities of genetic repair as humans
- Enzymatic Photolyase is used to repair genetic damage
- Yeast cells are typically able to repair genetic damage but certain strains have mutations that prevent them from performing DNA repair
- Yeast that is unable to repair damage usually dies through apoptotic pathways
- Yeast is often taken to space on ISS missions to show how radiation influences individuals over time
- This experiment shows that though genetic damage may occur in the short run, periods of

rest allow the continuation of normal metabolic activity as genetic damage is reversed over time.

**Radiation therapy**

- Mucositis (Adverse effects on the body due to radiation therapy)
- Radiation therapy can interfere with the cellular growth of epithelial cells, causing changes to normal development and cell death (apoptosis)
- Radiation slows cell division in the oral mucosal epithelium resulting in further complications in the treatment of cancer
- Radiation is typically administered daily from Monday to Friday with only a two day rest period
- This experiment demonstrates that daily doses prevent genetic mutation but also result in the immediate change in normal cellular activity.
- Cellular growth and development is drastically impaired, causing adverse effects on the healthy cells of the body

**No Limit Threshold Theory**

This study has shown that minimal levels of radiation, provided there are periods of rest from the dosages, have no adverse long term effects on the body and in some cases may aid in cellular regeneration

○ Increasing the limit for acceptable radiation dosages may therefore be an acceptable method to increase the rate of advances in radiation research without risking the health of individuals.

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