The impact of ultraviolet radiation on *Saccharomyces cerevisiae* survival

Chen, S., Lee, R., Oh, H, Preston, C.

Abstract

Liquid cultures of the yeast, *Saccharomyces cerevisiae*, were irradiated with the Mineralight® short wave ultraviolet lamp model UVS-11. The objective of the study was to determine the effect of irradiation of UV radiation on *S. cerevisiae*. The study included experiments where densities of 2.54×10^{-7} cells/mL and 3.56×10^{-7} cells/mL of yeast were irradiated for 5, 15 and 35 minutes. Then, with the use of a hemocytometer, we counted the cells within the grid. The population densities were based on cell counts per volume of yeast culture. The survival ratio was calculated by dividing final population density over initial density. While there was a trend for the population to drop according to time exposed, it was not statistically significant, and our results were inconclusive. We were not able to support the hypothesis that exposure to ultraviolet light will reduce the population density of yeast, and we fail to reject our null hypothesis.

Introduction

Saccharomyces cerevisiae is a species of yeast that is commonly used for brewing and baking. They are eukaryotic microorganisms classified in the kingdom Fungi. Yeast cells can grow in two forms: diploid or haploid (Mucka *et al*. 2010). *S. cerevisiae* usually reproduce asexually by an asymmetric division process called budding; however, under stressful conditions, the diploid cells of yeast (the preferred form) can pursue sporulation and produce haploid spores via meiosis. *S. cerevisiae* can grow aerobically on glucose and maltose but fail to grow on lactose (Yu *et al.* 2011).

Along with eleven other microorganisms from all three domains of life (bacteria, eukaryota, and archaea), a sample of living S. *cerevisiae* was included in the Living Interplanetary Flight Experiment (LIFE). It is a 34-month interplanetary mission to Mars' moon Phobos and tests whether microorganisms can survive outside of Earth's protective magnetosphere. It tests for the possibility of transpermia, which claims that life travels between

planets via meteoroids, and may support the theory that origin of terrestrial life came from outer space (Project LIFE Experiment, 2011).

Scientists have been testing the possibility of transpermia in laboratory settings. In a study by Horneck *et al.* (2010), they tested the effects of galactic cosmic radiation and solar UV radiation on viruses, bacterial cells and fungal spores in hopes to find how they would survive in space. This motivated us to investigate the effects of irradiation on *S. cerevisiae* cells using ultraviolet radiation since it was one of the microorganisms chosen to be part of LIFE.

There have been conflicting opinions on the effects of UV light irradiation on *S. cerevisiae*.

Previous experiments such as one done by Svihla *et al.* (1960) showed that the irradiation of cells with ultraviolet radiation lead to effects such as mutation and cell death. However, some literature suggested that ultraviolet light not only promoted cell growth (Gurwitsch and Gurwitsch 1934), but also released proliferation-promoting intercellular hormones, which stimulate growth (Sperti *et al.* 1937).

In our experiments, we compared the survival ratio of *S. cerevisiae* cells over time to provide support for our alternate hypothesis, which stated that increasing time exposure to constant intensity ultraviolet light will decrease the population density of *S. cerevisiae.* Consequently, our null hypothesis stated that increasing time exposure to ultraviolet light will increase the population density of *S. cerevisiae* or have no effect.

Methods

Preparation

We used 40 mL of the *Saccharomyces cerevisiae* haploid strain from the Department of Biology at the University of British Columbia in all experiments. Room temperature was 24°C in the lab and 23.5°C in the dark room. The light intensity in the lab was 558 lux, whereas it was 6 lux in the dark room. We sterilized the opening of the tube containing yeast culture using the

alcohol lamp before pouring yeast cultures into a 125 mL Erlenmeyer flask. Then, we diluted the culture to 100 mL with standard medium. We added 2 drops of methylene blue stain to differentiate between living and dead cells (only dead cells stained blue). In addition, we labelled the petri dish according to its type of treatment (see Figure 2).

Calculating Initial Population Density of the Yeast Culture

We pipetted 10 mL of the diluted yeast culture onto a hemocytometer under a Zeiss Axiostar compound microscope (see Figure 3). Under 40x magnification, we counted the number of cells on the grid of the hemocytometer. We only included cells that were touching the top and right boundaries of the grid (see Figure 1).

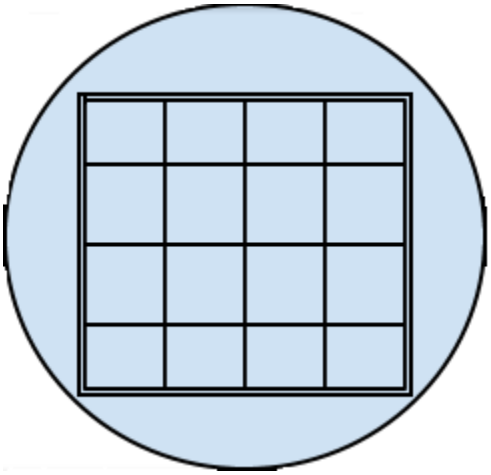

Figure 1: Drawing of grid of the hemocytometer under microscope

Population density of the yeast culture was calculated from cell counts using equation 1 below.

$$\text{Population density} = \frac{\text{Average of number of cells}}{\text{volume}} = \frac{\text{Average of number of cells}}{4 \times 10^{-6}\ \text{mL}} \quad \text{(eqn.1)}$$

If the value of population density was higher or lower than 3 to 4 x 10^{-7} cell/mL, we had to dilute or concentrate the yeast culture, respectively. We kept 50 mL of the yeast culture from the first dilution, and we diluted it to 100 mL. Then, we took 10 mL of the yeast culture and counted the

cell numbers under a Zeiss Axiostar compound microscope using a hemocytometer (see Figure 4).

Preparing the Samples and Controls

We pipetted 4000 microliters of yeast culture into each of eighteen petri dishes. We had three replicates per each treatment and controls for each of them.

Irradiation of UV radiation on Samples

We placed nine petri dishes randomly inside the box with the Mineralight® short wave ultraviolet lamp model UVS-11 atop and nine petri dishes outside the box in the dark room. We then exposed all experimental samples to ultraviolet light in a dark room for 5 minutes, 15 minutes, and 35 minutes (see Figure 5). Meanwhile, we exposed controls to air in the dark room. We started timing once the ultraviolet lamp was turned on. When the time passed, we quickly turned off the UV light within 30 seconds, took out the samples, and turned the light back on.

Calculating Final Population Density of the Yeast Culture

We measured the final population by counting cell numbers under the hemocytometer. 10 microliters was used for each measurement. We calculated population density for the initial and final populations using equation 1.

Calculating Survival Ratio

The ratio of yeast culture survival was calculated with equation 2.

$$\text{Survival Ratio} = \frac{\text{Final population density}}{\text{Initial population density}} \quad \text{(eqn. 2)}$$

Statistical Methods

We calculated 95% confidence intervals of final population density for each petri dish. We graphed the data with 95% confidence intervals for both experiments.

Figure 2: Photo of our petri dishes labelled. Experimental replicates are on the left, while controls are on the right.

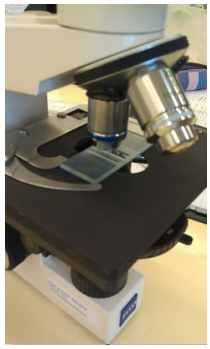

Figure 3: Photo of a hemocytometer under a Zeiss Axiostar compound microscope.

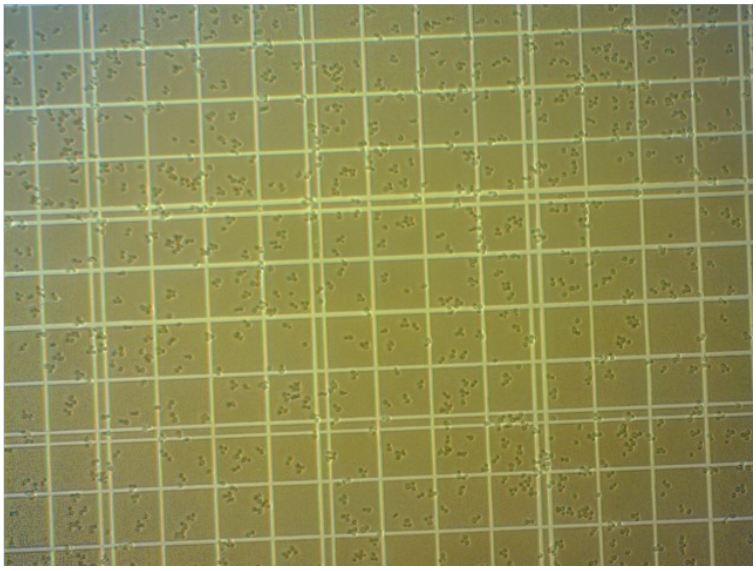

Figure 4: Photo of cells in the grid of hemocytometer

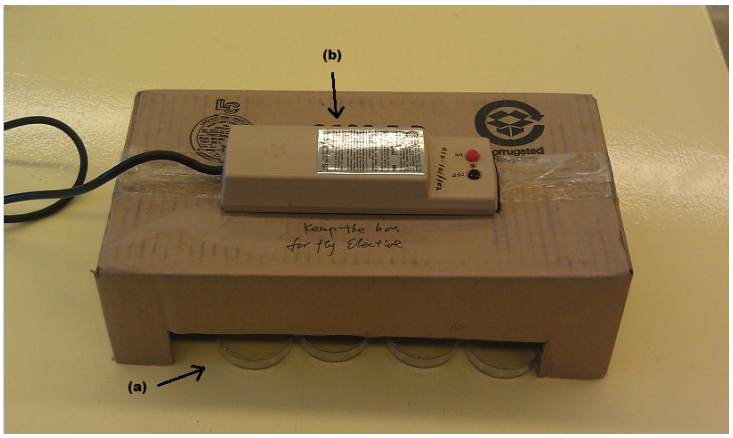

Figure 5: Photo showing the setup of the experiments in which yeast cells were irradiated with ultraviolet radiation. (a) are the petri dishes containing the yeast cells, and (b) is the ultraviolet lamp placed over the hole of the cardboard box.

Results

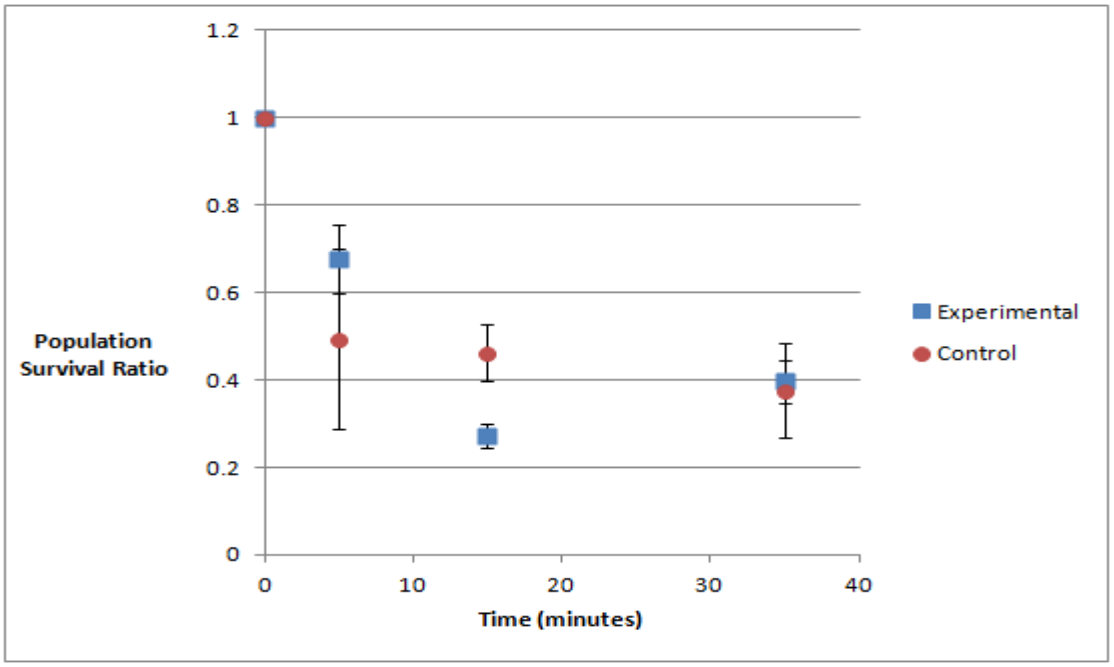

Figure 6: Mean values of 2.54 x 10-7 cells/mL *Saccharomyces cerevisiae* at 5, 15, and 35 minutes of Trial 1. 95% confidence intervals included.

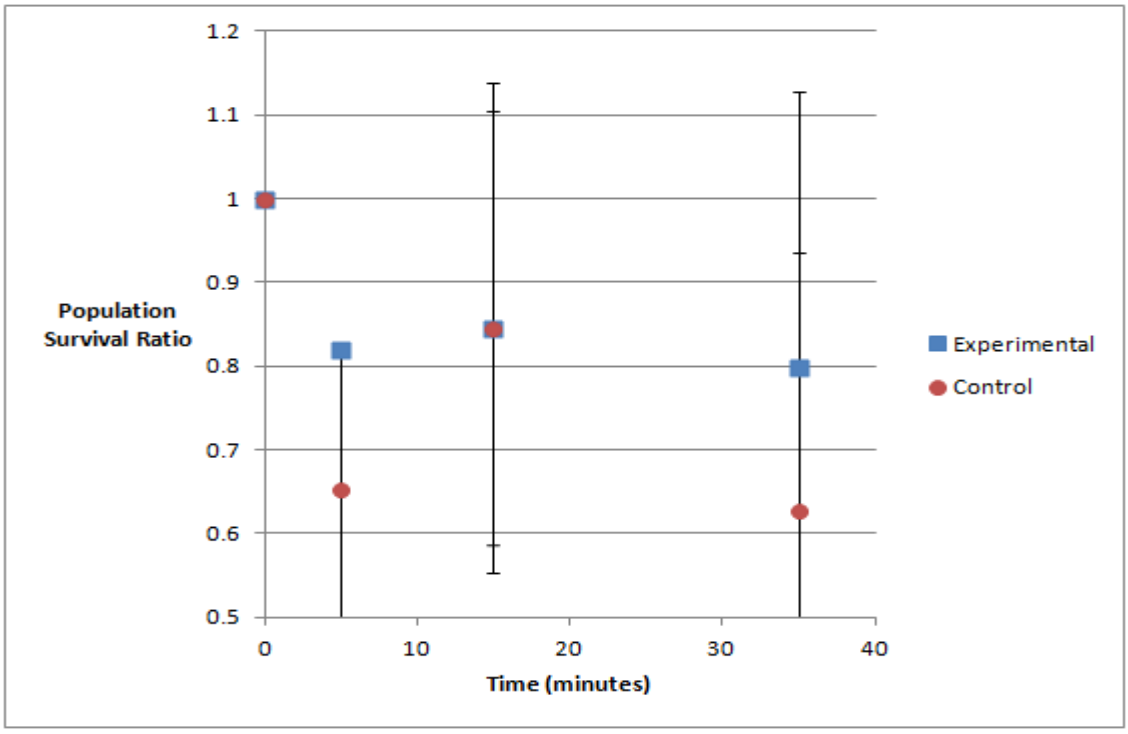

Figure 7: Mean values for cultures of 3.56 x 10-7 cells/mL *Saccharomyces cerevisiae* at 5, 15, and 35 minutes of Trial 2. 95% confidence intervals included. Note that for the data at time 15 min, the experimental and control points are at roughly the same spot.

Two experiments were conducted using continuous ultraviolet light. In each experiment, a scatter plot was created to determine significance with 95% confidence intervals. Control experiments were carried out alongside to allow for comparison.

Figure 6 shows the effect of UV light on *S. cerevisiae* in Trial 1. The graph shows a single significant data point at the 15 minute mark, as the 95% confidence intervals for the experimental and control do not overlap. A decrease appears in the control groups with increasing in time. The experimental groups appear to be random. At 5 minutes, the population density is the largest. However, at 35 minutes, the population density is higher than at 15 minutes. Therefore, it is not a decrease pattern as shown by the control groups.

Figure 7 shows the effect of UV light on *S. cerevisiae* in Trial 2. The graph demonstrates an overlap of 95% confidence intervals at all times. There is no apparent trend for the control groups. Unlike in Trial 1, the population density for control group at 15 minutes is highest. On the contrary, the experimental groups appear to have a stagnant linear pattern.

Discussion

We fail to reject our null hypothesis and fail to support our alternate hypothesis because the results were not statistically significant. However, the following trends were observed. At 15 minutes in Trial 1, the sample's UV exposed population survival ratio was lower than that of control. More cells died per unit time exposed to UV light than in Trial 2 and the changes in the survival rate were statistically significant for some data points. Trial 2 had wildly varying results with a large margin of error, possibly due to counting error, as we had more people counting cells in Trial 2 than in Trial 1.

The data in Trial 1 at 15 minutes showed decrease in population survival ratio. This finding can be supported by previous irradiation experiments on *S. cerevisiae* like the one done by Svihla *et al*. (1960). They found that irradiation by ultraviolet radiation is harmful. Even though it shows an increase of population survival ratio at 35 minutes in Trial 1, the confidence intervals overlap with the control so the data are not significantly different.

Many possible errors lead to large confidence intervals and varying data. One of the possible errors could be that we added the methylene blue stain during our preparation work, before UV radiation exposure. Although methylene blue stain is important for distinguishing between dead and live cells, we added it in early in our experiment and exposed it to the *S. cerevisiae* cells for almost 3 hours. Prolonged exposure of methylene blue can be very toxic, which explains the low population survival ratios of the controls (C. Pollock, personal communication).

Another reason behind the largely varying data in Trial 2 could be not shaking the yeast culture in the petri dishes after exposure to UV radiation. After 5, 15 and 35 minutes of being stationary, yeast cells in the petri dishes could have sunk down to the bottom. Therefore, the cell numbers that we counted would have been lower.

It is possible that our hypothesis is incorrect, and UV light of the intensity used is not necessarily harmful. Some literature suggested that ultraviolet light not only promoted cell

growth (Gurwitsch and Gurwitsch 1934), but also released proliferation-promoting intercellular hormones that stimulate growth (Sperti *et al.* 1937). Some of our experimental variation may be due to the fact that different replicates were exposed to different intensities of UV light, with some being of the right intensity to induce cell death, and others merely causing the yeast to release these substances.

Another possible source of error was that the mechanism by which the yeast cells died was not, in fact, the UV light. Given that the control groups also showed densities lower than the initial population, and in some cases, the experimental replicates, it could be that exposure to air had a negative effect on population density. In order to control this, we would need to put the control group in a box similar to the one experimental subjects were in, to ensure that the airflow across the replicates is roughly equal.

In addition, the medium in which yeast cells resided could have been a factor in our inconsistent results. In Trial 2, we had to dilute the yeast more and perhaps during the process of adding the composition of the new media the yeast cells could have been affected. According to Woodrow and Fulmer (1927), UV light does not kill the yeast cells directly but increases the toxicity of the media by changing its composition. They found that UV light indirectly killed the yeast cells due to media. Therefore, some samples' composition of media in Trial 2 may have changed and killed more cells due to the increase in toxicity and produced different results from the data in Trial 1.

Another possible explanation for our results could be biological variation and the low number of replicates. We only used three replicates for each exposure in the experiments, and, as can be seen by the large 95% confidence interval, a high degree of variance was present. It is possible that with a larger number of replicates, we would see a trend that is currently obscured by the sheer magnitude of variation present.

Conclusion

A decrease in population density of *S. cerevisiae* cells was observed, but we cannot attribute this outcome to being a consequence of ultraviolet radiation exposure. The data from our results are inconclusive as we are unable to reject our null hypothesis or support our alternate hypothesis that exposure to UV light would cause a decrease in population.

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