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A Study on Yeast Using the Photoreactivation Process to Repair the Pyrimidine Dimer Mutations

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Abstract: Sunlight has an indispensable importance for living things in nature [1-3]. However, the direct absorption of UV will lead to the formation of pyrimidine dimers between adjacent pyrimidines in DNA strands usually in the form of cyclobutene pyrimidine dimers (CPDs) and pyrimidine (6-4) pyrimidone photoproducts (6-4PPs) which causes great damage [4-6]. A DNA repair system, known as photoreactivation, can effectively repair the dimers using photolyase [7-9], which has currently been found in plants, prokaryotic and eukaryotic cells [10-12]. This study was carried out to determine whether photolyase DNA repair can be observed in yeast. Several yeast Petri dishes were treated with ultraviolet radiation, different treatments were then added to them, and the colonies were counted after culturing, hence verifying that yeasts can use the photoreactivation process.

Keywords: Photoreactivation; DNA repair; Photolyase

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1. Introduction

The yeast *Saccharomyces cerevisiae* (*S. cerevisiae*) is a unicellular eukaryote that has haploid and diploid forms. *S. cerevisiae* is widely distributed in nature, and its growth rate is significantly affected by environmental changes, of which temperature and pH value are the two main aspects [13,14]. It is acidophilic and the optimal growth temperature is 28–30℃. Commonly used as a model organism for studying eukaryotes, *S. cerevisiae* has many identical structures with animal and plant cells and is easy to culture. It is also the most commonly used biological species in fermentation, as people often use it as the main strain for alcohol production and juice fermentation [15,16].

2. Materials and Methods

2.1. Selection of dilution of the yeast culture and the UV-C exposure time

Sixty-three Petri dishes of *S. cerevisiae* were plated with a dilution of 1/10 and 1/100, respectively. They were located under the same UV-C light intensity and other environmental factors. The most suitable UV-C exposure time was determined to achieve LD_{50} (the dose that kills 50% of the test population), ensuring the damaged cells were not too few and not representative due to short duration, or too many due to long duration. Hence, 9 of each dilution were removed every 30 s including time point 0 followed by colonies counting after visible colonies formation. The data was plotted to fit a linear model that indicated the optimal dilution and UV-C LD_{50}, which were 1/100 and 1.5 min, respectively.

2.2. The UV-C radiation and light exposure experimental procedure

A total of 30 yeast dishes with a dilution of 1/100 were distributed under 3 experimental conditions evenly: (1) non-UV radiated yeasts (nonirradiated group); (2) UV-radiated and kept in the dark after radiation 1.5 min (irradiated dark group); and (3) UV-radiated and exposed to the sun after radiation 1.5 min (irradiated light group). These Petri dishes were all placed in the same environment of 25℃, 70% relative humidity, and with the same light irradiation intensity and other confounding factors. Each group had 10 replications. All the Petri dishes were inserted into an incubator to allow living cells for colony formation and counting.

2.3. Statistical analysis

The initial data was plotted to fit a linear model in order to select the optimal dilution and calculate the UV-C LD_{50}. One-way ANOVA and Tukey HSD in the RStudio were used to observe whether there is statistically significant photoreactivation.

In the ANOVA, the null hypothesis was assumed to be no difference between living cell count across the treatment. The outliers in the data were removed and checked with diagnostic plots.

In the Tukey HSD test, the data were made in pairs and divided into 3 categories: (1) the irradiated light and irradiated dark; (2) the nonirradiated and irradiated dark; (3) the nonirradiated and irradiated light. Three null hypotheses were made:

(1) There is no difference between the mean cell count in the irradiated light and irradiated dark groups.
(2) There is no difference between the mean cell count in the nonirradiated and irradiated dark groups.
(3) There is no difference between the mean cell count in the nonirradiated and irradiated light groups.

3. Results

Figure 1 showed the number of cells at 1/10 and 1/100 dilutions at different exposure times. There were hundreds of colonies per plate with 1/10 dilution which were all bigger than that with 1/100 dilution. There were less than 200 cells in all of the 1/100 dilution cultures.

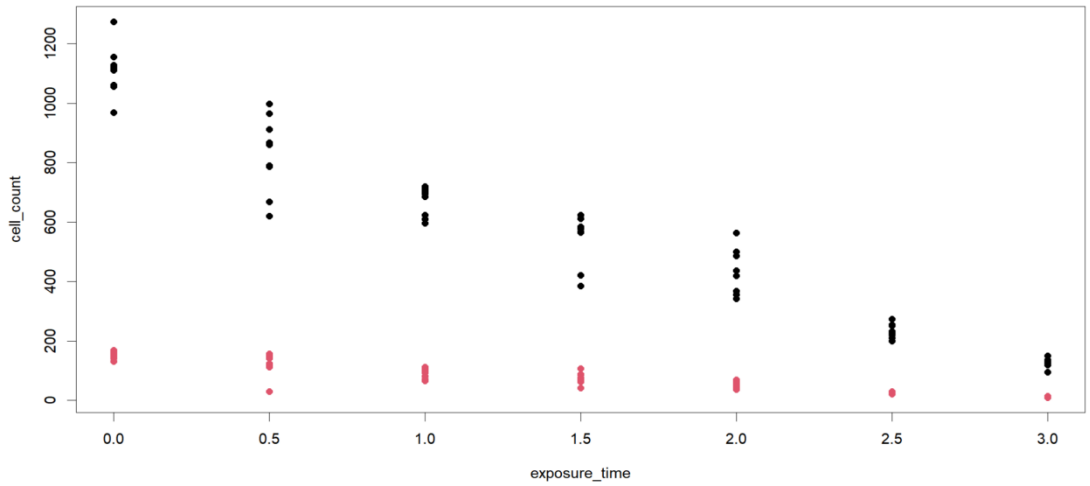

Figure 1. The scatter plot of the 1/10 and 1/100 dilution cultures data at different exposure times.

Figure 2 showed the regression line of the changes in cell count with exposure time where half of the cell population died at 1.5 min. In other words, the LD_{50} is 1.5 min.

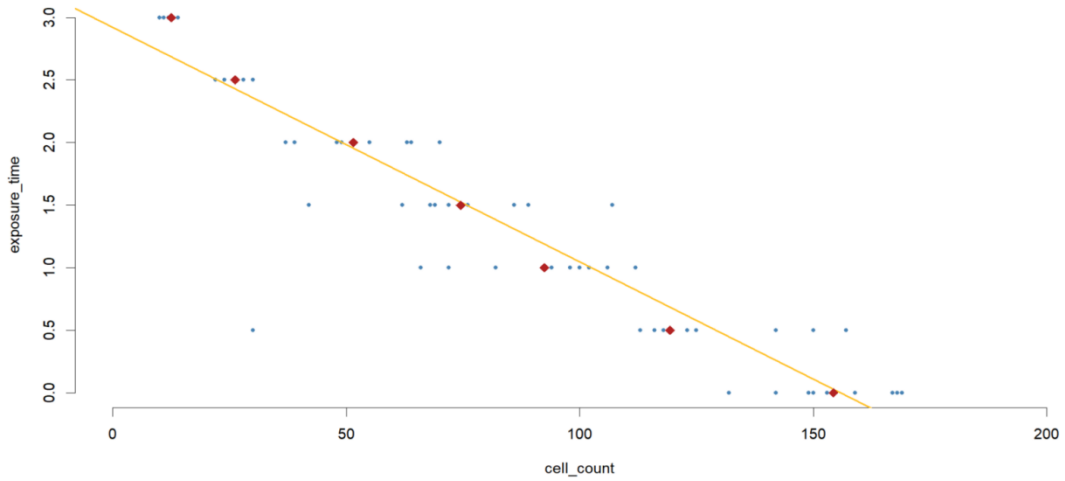

Figure 2. The plot of the regression line showed the changes in cell count with exposure time.

The data of cell numbers in the nonirradiated, irradiated dark, and irradiated light groups were plotted with a boxplot in RStudio. Almost all data were concentrated below 200. However, two plates in each treatment with an unusually high number of colonies were observed.

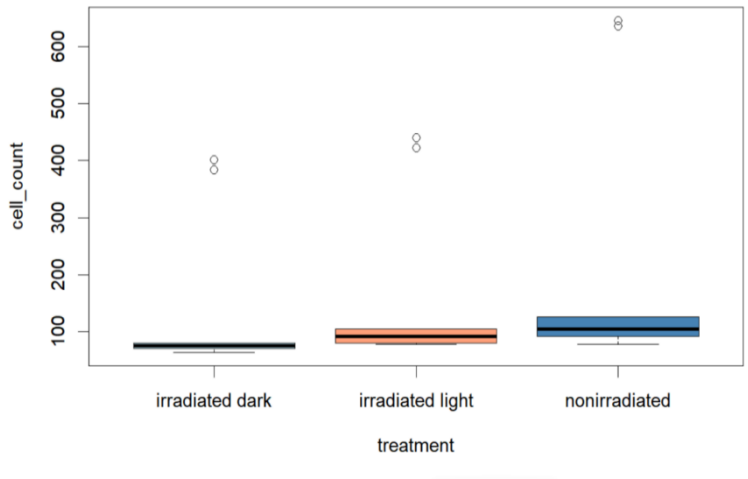

Figure 3. Distribution of yeast colony number in box plot under three environmental conditions.

The data with outliers removed were plotted with a boxplot. As shown in **Figure 4**, the number of living yeast in the nonirradiated group was the highest, whereas the irradiated light group had more living yeast colonies than the irradiated dark group, but both groups had lesser living yeast colonies than the nonirradiated group.

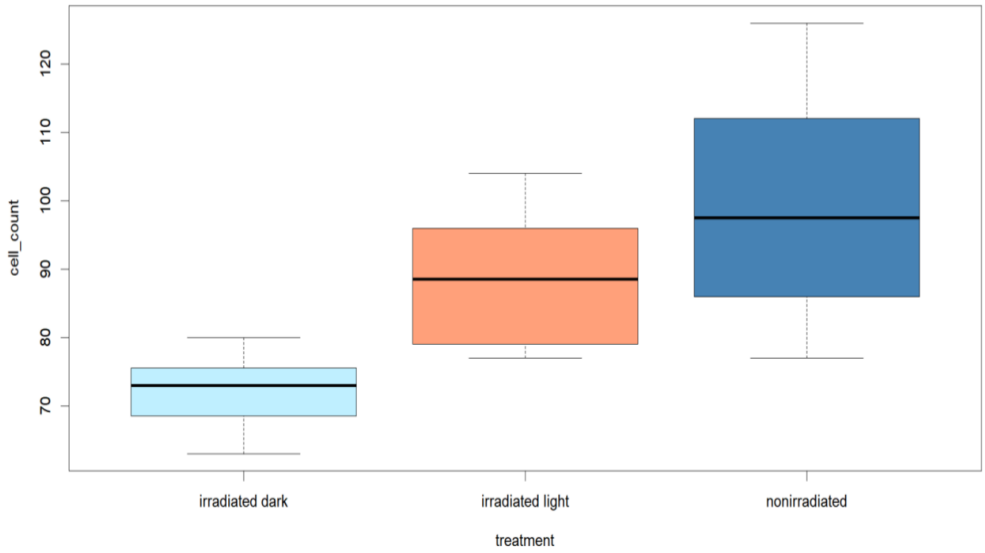

Figure 4. Distribution of yeast colony number in boxplot under three environmental conditions after removing the outliers.

The average number of living yeast in each treatment category was calculated. According to **Table 1**, the nonirradiated group had the highest cell count while the cell count in the dark environment was the least.

Table 1. The mean number of cells calculated in each treatment category

Treatment	Cell count
Irradiated dark	72.125
Irradiated light	88.500
Nonirradiated	99.250

To calculate the mean squares, the *F*-statistic, and the *P*-value, the ANOVA was used (results shown in **Figure 5**). The *P*-value found was 0.000654.

```
            Df Sum Sq Mean Sq F value   Pr(>F)
treatment    2   2985  1492.6   10.61 0.000654 ***
Residuals   21   2954   140.7
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Figure 5. The ANOVA of data analysis in RStudio.

Figure 6 shows the Tukey HSD test analysis of the data. The Tukey HSD test showed the *P*-values of the 3 categories were 0.03, 0.00047, and 0.19, respectively.

```
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = cell_count ~ treatment, data = y.fix)

$treatment
                                    diff       lwr      upr     p adj
irradiated light-irradiated dark 16.375  1.426694 31.32331 0.0301853
nonirradiated-irradiated dark    27.125 12.176694 42.07331 0.0004652
nonirradiated-irradiated light   10.750 -4.198306 25.69831 0.1898423
```

Figure 6. Tukey HSD test of data analysis in Rstudio.

In **Figure 7**, the 95% confidence intervals of the test statistics were plotted, and the intervals of nonirradiated-irradiated light included 0.

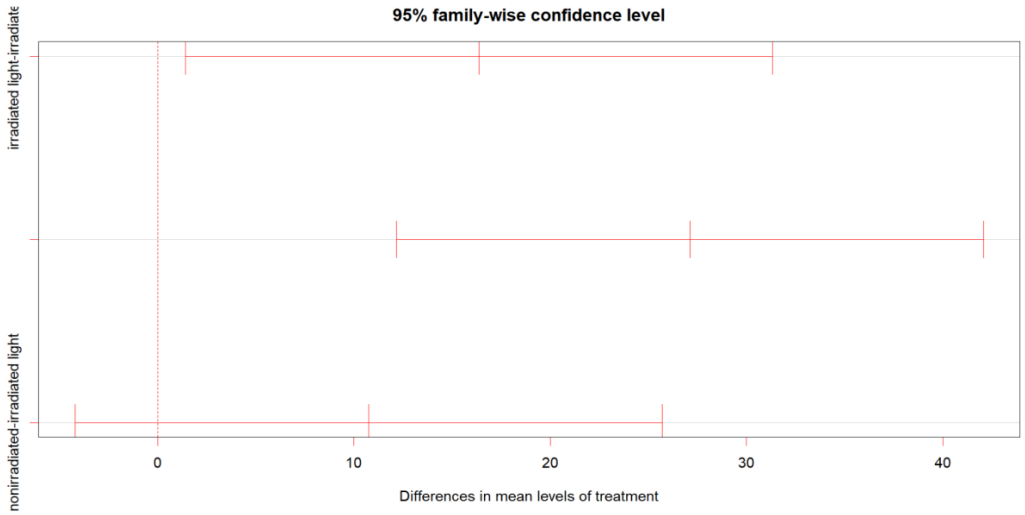

Figure 7. The plot of the 95% confidence intervals of the test statistics.

In **Figure 8**, the 'Residuals vs Fitted' plot showed a red line close to being horizontal at 0, and some of the points were randomly and evenly distributed. In the 'Normal Q-Q' plot, a line of best fit was drawn from the points. The 'Scale-Location' plot showed a steeper red line as compared to the 'Residuals vs Fitted' plot. The 'Constant Leverage: Residuals vs Factor Levels' plot showed a near horizontal red line as there are some points further than 2 units from the 0.

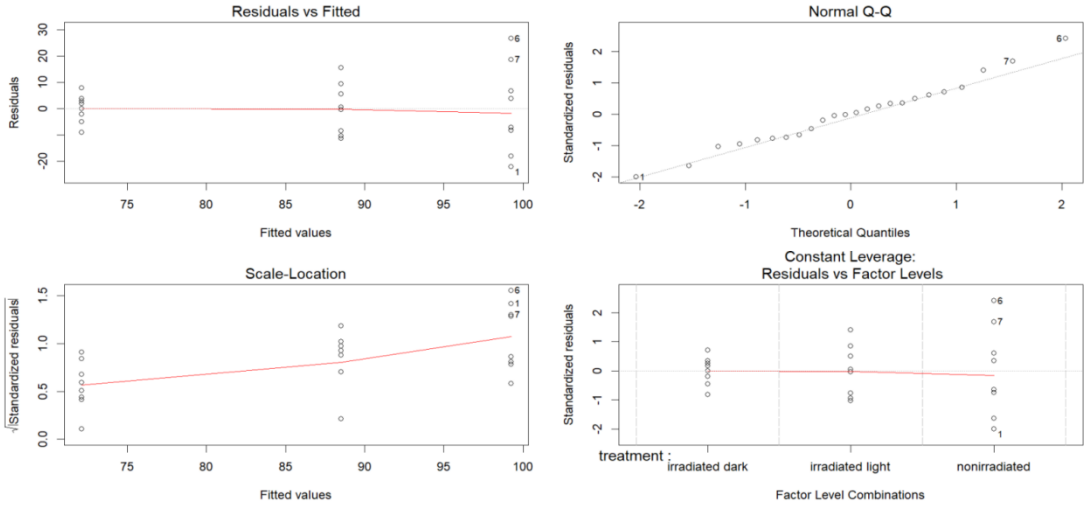

Figure 8. The diagnostic plots to check the assumption of ANOVA.

4. Discussion

4.1. Analysis of the distribution of yeast colony number in the boxplot

According to **Figure 2** and **Table 1**, the number of living cells in both groups after irradiation was lower than that in the nonirradiated group, which indicated that UV irradiation affected the cells. Meanwhile, the boxplot intuitively showed that light, such as sunlight, helps promote the DNA repair of *S. cerevisiae*. However, the efficiency of DNA repair in the dark environment is comparatively low. Hence, it is deduced

that the DNA repair degree of *S. cerevisiae* in light is higher than that in dark environments.

4.2 Analysis of ANOVA

According to ANOVA, the *P*-value is 0.000654 which is much smaller than the significance level $\alpha = 0.05$. Also, the assumption of ANOVA was checked by diagnostic plots shown in **Figure 6**. Therefore, the null hypothesis is rejected, and it is concluded that there is some relation between the living cell count and the treatment. Hence, it is preliminarily found that *S. cerevisiae* had a certain photoactivated DNA repair mechanism.

4.3. Analysis of Tukey HSD test and the 95% confidence intervals

According to the Tukey HSD test, the *P*-value of the nonirradiated-irradiated light groups is 0.19 which is bigger than the significance level $\alpha = 0.05$, and the intervals of nonirradiated-irradiated light groups included 0, making it statistically insignificant, so the null hypothesis is not rejected. On the other hand, the *P*-value in the irradiated light and the irradiated dark groups is 0.03 which is smaller than $\alpha = 0.05$, so the null hypothesis is rejected and there is some difference between the irradiated light and the irradiated dark groups. From this perspective, it is concluded that there is little difference in the viable count between the nonirradiated and irradiated light groups despite the irradiated light group having UV-damaged DNA followed by a period of sunlight exposure. However, the DNA repair rate in the irradiated light group is significantly higher than that in the irradiated dark group, which is consistent with the fact that yeast preferentially uses photolyase to repair non-transcribe strands of active RNA polymerase II and III transcribed genes instead of nucleotide excision repair (NER), leading to higher efficiency in DNA repairing [17-19].

4.4. Limitations of the study

Although the confounding variables of the different groups were controlled to be identical, there may still be variations that existed, for example, the inconsistency in the state of the ultraviolet lamp used in each group, the intensity of light, and the initial cell activity of different yeast groups, which are likely reflected in the outliers in **Figure 3**. Furthermore, some deviations in the statistical analysis are shown as the outliers in 'Residuals vs Factor levels' in **Figure 8**, which makes the ANOVA slightly imprecise. The sample size in subsequent experiments should be increased to reduce the chance of variations in experimental results [20].

5. Conclusion

In this study, there is no difference between the nonirradiated and irradiated light groups, but there is a significant difference in DNA repair rate between the irradiated light and irradiated dark groups, which indicated the efficient DNA repair mechanism of photolyase after a period of sunlight exposure despite DNA being damaged by UV. The photolyase DNA repair mechanism is observed in yeast, and it is concluded that yeast can use the photoreactivation process which uses photolyase and the light energy to repair the pyrimidine dimer mutations.

Disclosure statement

The author declares no conflict of interest.

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