

## Survival of *Escherichia coli* to UV Irradiation During Exponential and Stationary Phases of Growth

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**In their natural environment, bacteria most often live in nutrient-limiting conditions and are exposed to various stresses such as UV radiation. As a result, they have evolved a number of protective mechanisms, such as the SOS response, to prevent DNA damage. Bacteria in exponential, stationary, or death phase may be prone to UV damage to different degrees depending on the fidelity and the accuracy of the DNA repair systems. To investigate how the DNA repair mechanisms are operating in *Escherichia coli* during log and stationary phase, survival of the cells in response to UV irradiation was assessed. In stationary phase, *Escherichia coli* demonstrated increased resistance to UV exposure compared to cells in exponential phase. This indirectly suggests either an up-regulation of DNA repair mechanisms during stationary phase or a decreased rate of DNA replication, which allows more time for DNA repair.**

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Bacteria in their natural environment are often exposed to growth-limiting conditions such as nutrient starvation. In response to nutritional deficiencies, the level of the intracellular signaling molecule ppGpp increases (3). ppGpp is an alarmone that binds to the RNAP complex and regulates expression of a number of genes including the stationary phase sigma factor  $\sigma^S$  encoded by *rpoS* (2). One of the genes upregulated by  $\sigma^S$  is *dps*, which encodes a DNA binding protein and has been reported to be important in oxidative stress resistance (1, 3). UV radiation induces intracellular oxidative damage through the production of reactive oxygen species. Therefore, cells in stationary phase that have upregulated their  $\sigma^S$  dependent genes would be expected to be less prone to oxidative damage caused by UV radiation.

It has also been documented that expression of some DNA repair mechanisms is upregulated in stationary phase. In particular, the SOS response is induced in stationary phase even in the absence of DNA damage, its endogenous signal (6). SOS is a global response in which the cell cycle is arrested and DNA repair and mutagenesis systems are induced (5). A major part of this response is the derepression of over 20 genes under the control of the LexA repressor, which are mostly involved in DNA repair. Included in the LexA regulon are the recombination and repair genes *recA*, *recN*, and *ruvAB*; nucleotide excision repair genes *uvrAB* and *uvrD*; and the error-prone DNA polymerase genes *dinB* (Pol IV) and *umuDC* (Pol V; 5).

This experiment examines the effects of UV on the survival of cells in log and stationary phase. *Escherichia coli* SMR4562, which is genetically identical to *E. coli* FC40 but was constructed independently, was used in order to examine this phenomenon. *E. coli* SMR4562 has a slightly leaky Lac<sup>-</sup> phenotype which is not sufficient to allow the bacterium to grow on lactose-containing media (7). *E. coli* FC40 has been used extensively to study adaptive mutation because it readily reverts back to a Lac<sup>+</sup> phenotype when grown in a medium that contains lactose as the sole carbon source. It is hypothesized that stationary phase cells exposed to UV will have a higher rate of Lac<sup>+</sup> revertants than those in the log phase. Two possible explanations exist. Firstly, as described earlier, stationary phase cells have committed a large portion of their cellular resources towards increasing survival in adverse conditions such as UV exposure and nutrient starvation. Secondly, the stationary phase cells have increased mutagenesis due to activation of the SOS system as described above. In addition to the SOS system which is upregulated in response to UV damage in both exponential and stationary phase cells, the stationary phase cells have  $\sigma^S$ -dependant repair mechanisms expressed prior to UV exposure. This increases their ability to survive UV radiation.

In this study, it was observed that cells in stationary phase were generally more resistant to UV-induced mutation than cells in log phase. We were, however, unable to investigate the rate of UV-induced mutation through Lac<sup>+</sup> reversion as our starter culture turned out to contain reverted Lac<sup>+</sup> bacteria. This is a side effect of using *E. coli* SMR4562, which has a genetically unstable *lac*<sup>-</sup> mutation.

## MATERIALS AND METHODS

**Bacterial strains.** The strain used in this study was *E. coli* SMR4562, kindly supplied by Dr. Susan Rosenberg at Baylor College of Medicine in Texas. *E. coli* SMR4562 bears an F<sup>+</sup> plasmid with a *lacI/lacZ* fusion, which eliminates the coding sequence for the last four residues of *lacI* and all of *lacP* and *lacO* (5). *E. coli* SMR4562 cannot utilize lactose due to a +1 frameshift mutation that reduces the level of  $\beta$ -galactosidase to about 1% of the normal level. However, it may revert to Lac<sup>-</sup> if the proper mutation is introduced.

**Media.** Bacteria were cultured in M9 minimal salts liquid media supplemented with 0.5 mg/L thiamine and 0.1% glycerol. *E. coli* SMR4562 Lac<sup>+</sup> revertant colonies were isolated on M9-agar plates containing 0.1% lactose and 0.5 mg/L thiamine. M9-agar plates containing 0.1% glycerol, 0.5 mg/L thiamine and 1.5% agar were used to plate control samples.

**Culturing methods.** *E. coli* SMR4562 bacteria were resuscitated from the culturette vial supplied by Dr. Rosenberg by streaking on a Luria broth (LB) agar plate. The LB plate was grown overnight at 37°C and isolated colonies were used for further experimentation.

**Adaptive mutation assay.** Twenty-five millilitres of M9+glycerol medium was inoculated and grown overnight at 37°C in a shaker at 150 rpm. The next morning 50 ml of M9+glycerol was inoculated with the overnight culture to an initial OD<sub>460nm</sub> of 0.12. The culture was sampled at 30-minute intervals over a period of 8.5 hours. At each time point the OD<sub>460nm</sub> was taken and the appropriate serial dilutions were carried out. Final plated dilutions ranged from 10<sup>-5</sup> to 10<sup>-7</sup>. Four M9+lactose agar plates were plated using a spread plate technique. Two of these plates were plated with a 10-fold higher dilution and irradiated at 2.1 mJ using UV Stratalinker™ 2400 (Stratagene). All the plates were incubated at 37°C and were checked daily for new colonies. Control plates were set up using a culture prepared in the same manner and parallel to the adaptive mutation assay. These samples were plated on M9+glycerol plates and incubated at 37°C only until colonies appeared.

**Survival curve.** Approximately 690 cfu were plated on M9+glycerol plates. The plates were irradiated at 0.3, 0.5, 1.0, 1.5, and 2.1 mJ and were incubated at 37°C until colonies appeared.

## RESULTS

Before initiating the experiment, *E. coli* SMR4562 was tested for growth on both lactose and glycerol M9 media. Growth on the glycerol plate was detected after two days of incubation at 37°C. There was no growth on the lactose plate after an equivalent time period, but revertants began to appear after three days.

Initially a growth curve was constructed to determine when stationary phase begins for *E. coli* SMR4562 grown in M9 minimal media (data not shown). Two trials of the experiment were carried out. The first was inconclusive due to excessive contamination and a lethal UV dosage; in the second experiment reversion frequency could not be examined due to the fact that colonies appeared in similar numbers on both the lactose and glycerol plates after two days of incubation. This was unexpected because *E. coli* SMR4562 was believed to have a Lac<sup>-</sup> phenotype. To resolve this anomaly, a colony from a glycerol plate was chosen and plated on an M9+lactose plate. After one day of incubation there was evidence of growth on the lactose plate, which is consistent with a Lac<sup>+</sup> phenotype.

The survival curve (Fig. 1) shows that there was a steady decrease in survival with increasing exposure to UV. Survival was described as the percentage of cells originally plated that survived the UV treatment. A UV intensity of 2.1 mJ was chosen for the remainder of the project because it showed an 11% survival rate, which would provide a meaningful means of comparison.

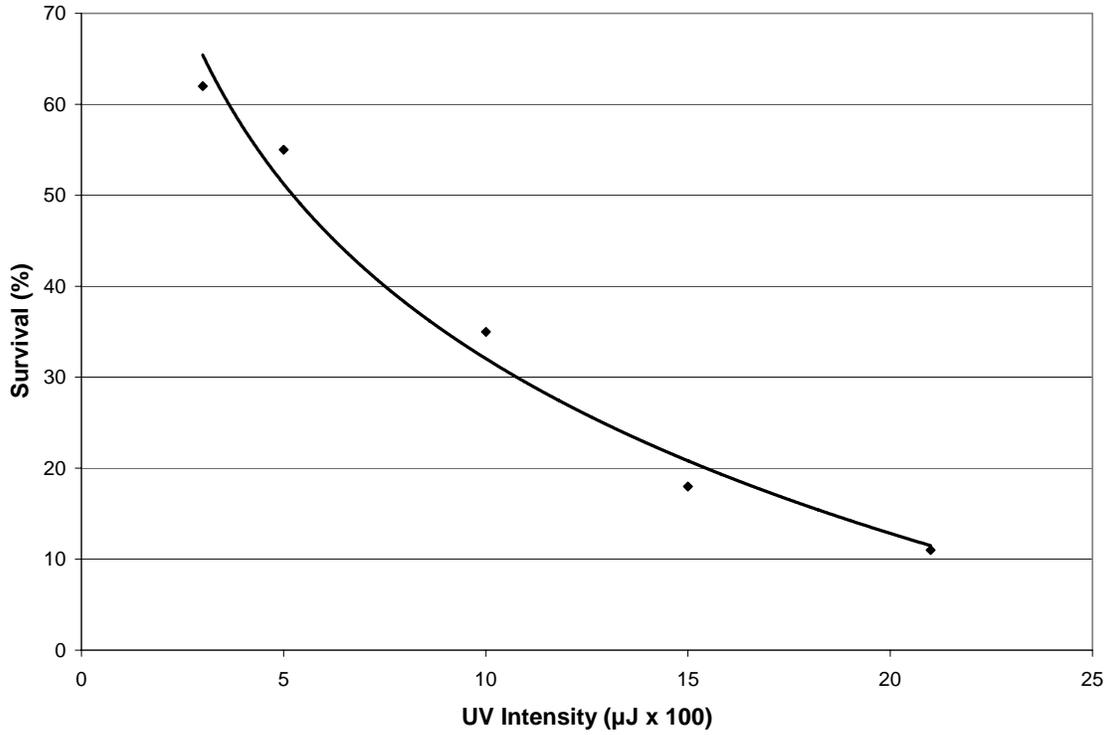
The growth curve (Fig. 2) was constructed based on turbidity measurements over 8.5 hours. There appears to be a short lag phase in the first 30 minutes followed by a fairly constant log phase until 6 hours, and finally a transition into stationary phase.

A second growth curve was constructed based on the plate counts (Fig. 3), which gave different results than those seen in Figure 2. The data from the glycerol, non-irradiated plates and the lactose, non-irradiated plates show that the culture did not enter log phase until approximately 3 hours, when the concentration in CFU/ml began to increase slowly. Based on the colony counts, no stationary phase could be detected.

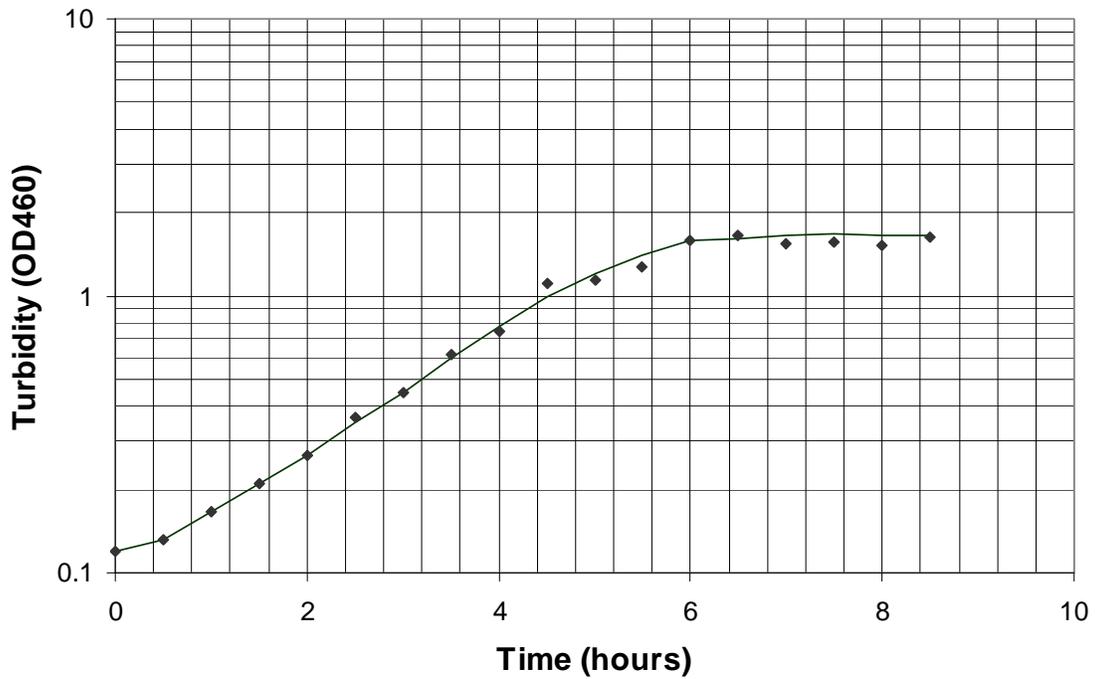
The irradiated lactose and glycerol plate counts showed that from 0 to 3 hours there was a gradual decrease in survival after UV exposure, followed by a period between 4 and 6.5 hours where the survival remained relatively constant. After 6.5 hours there was a steady increase in survival.

Figure 4 shows the ratio of the number of cells surviving the UV treatment to the total number of cells on the corresponding non-irradiated plates. It appears to have three regions; initially there is a steady decrease until 3 hours. Survival from 3.5 – 6.5 hours appears to be relatively constant, followed by a sharp increase in survival until 8.5 hours.

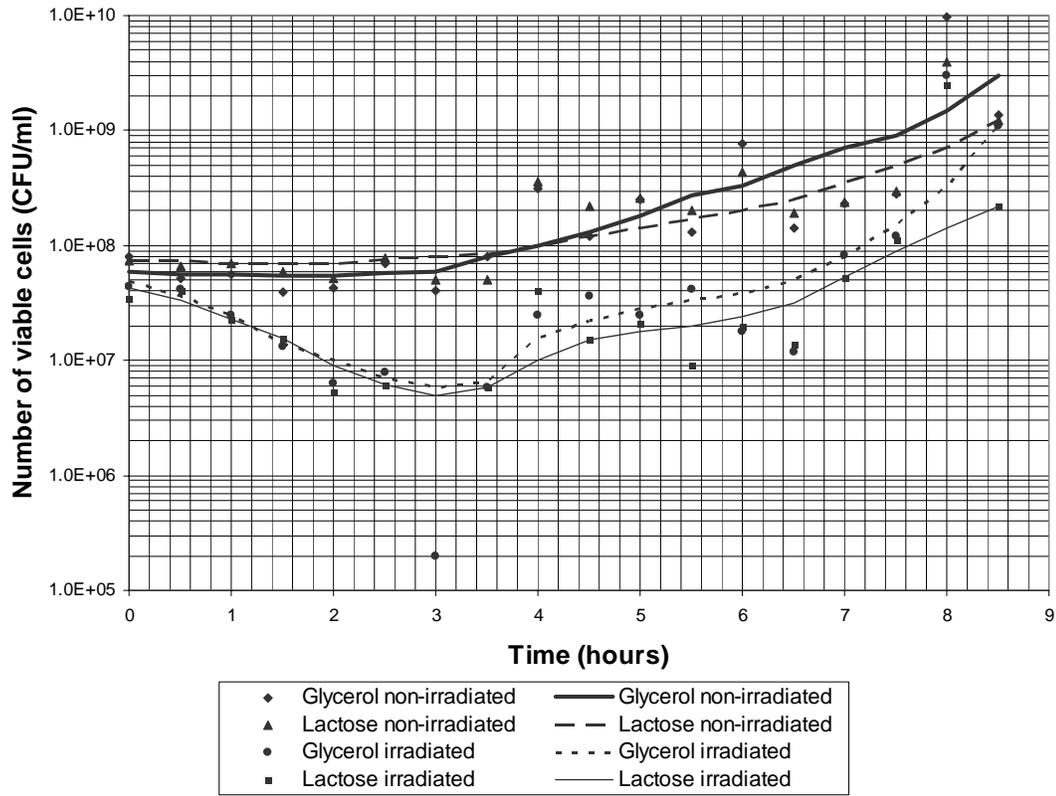
Figure 5 shows the relative frequency of survival of the bacteria grown on lactose plates and exposed to UV compared to those grown on glycerol and exposed to UV. The difference in survival between bacteria grown on lactose and glycerol for the first 2.5 hours was relatively constant, approximately 70 per cent. From 3 hours onward, the graph became very erratic.



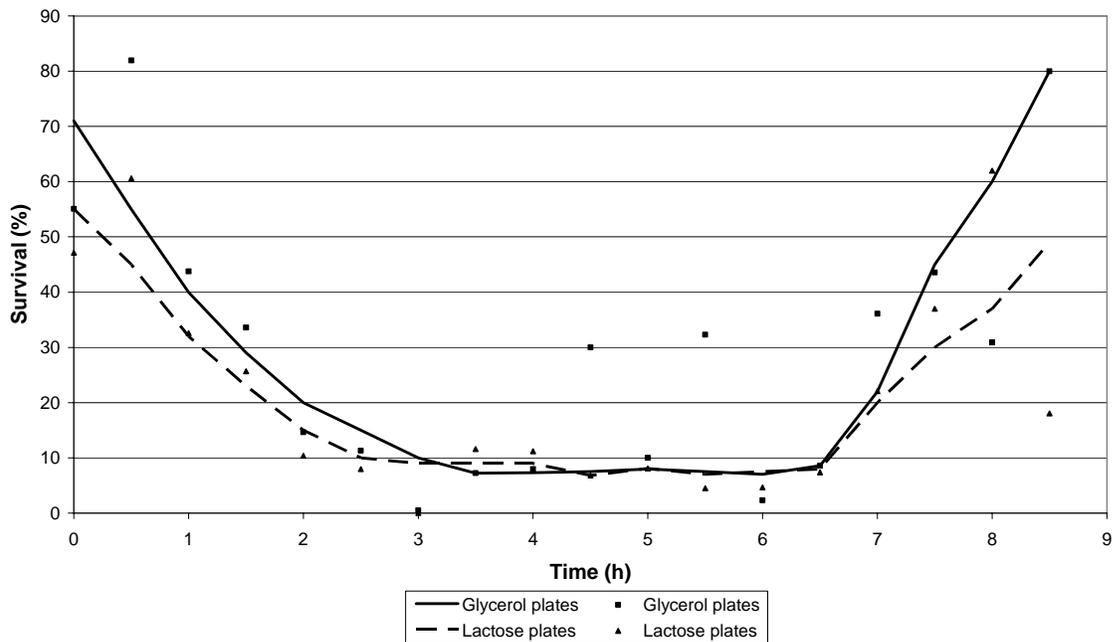
**Figure 1.** Survival curve of *E. coli* SMR4562 exposed to varying intensities of UV light (mJ)



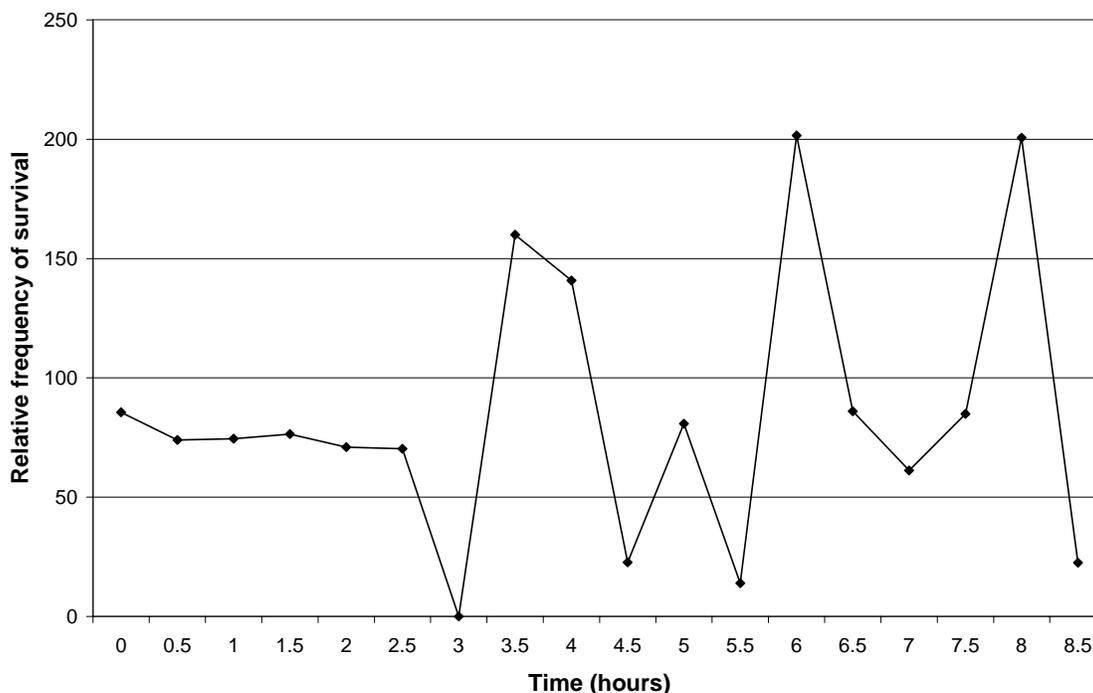
**Figure 2.** Growth Curve of *E. coli* SMR4562 grown in M9 minimal media with 0.1% glycerol and 0.5mg/L thiamine.



**Figure 3.** Growth curve of *E. coli* SMR4562 grown in M9 minimal media with 0.1% glycerol and 0.5 mg/L thiamine.



**Figure 4.** Percentage of plated bacteria surviving the UV treatment on both M9+Lactose and M9+Glycerol plates.



**Figure 5.** Relative frequency of survival of bacteria grown on lactose plates and exposed to UV compared to those grown on glycerol plates and exposed to UV

### DISCUSSION

In order to investigate the stationary phase in *E. coli* SMR4562, two growth curves were constructed, one based upon optical density at 460 nm and the other on bacterial numbers (cfu) (Fig. 2 and 3). The two graphs give conflicting results; the growth curve based on optical density showed an exponential phase from 0.5 hours – 6 hours and stationary phase from 6 hours onward, whereas the growth curve based on bacterial concentration did not show any evidence of stationary phase. Only the beginning of what seemed to be an exponential phase is apparent from 3 hours onward. Growth curves based on plate counts rely solely upon bacterial numbers to illustrate growth. Between 0-3 hours the concentration of bacteria remains approximately constant, whereas after three hours the data points vary slightly. This time point corresponds to the time when the dilution factor was increased and as a result, some of the plates were not statistically accurate (i.e. did not have 30 – 300 colonies). This led to uncertainty in the data, which is reflected in the ambiguous data points. On the other hand, turbidity values are also subject to error. Under the shift-up conditions used in this experiment, bacterial size increases before bacterial division commences. Therefore, as turbidity measurements are based on the scatter of light, increasing cell size can increase readings independent of cell concentration. Although the growth curve based on turbidity may have some inherent error due to this variable, it is unlikely that this has affected the entire growth curve therefore it is likely that this growth curve is more reliable and accurate.

Figure 4 describes the percent survival of the bacteria grown on either glycerol or lactose media that were exposed to UV radiation. In the first 3 hours there was a gradual decrease in survival. During this time period the bacteria were in exponential growth and taking advantage of the abundant nutrients and favourable growth conditions. Therefore, rather than directing resources towards survival they were directing a majority of their resources towards replication so the cells at this stage were more susceptible to UV damage.

An increasing resistance to UV damage was detected after 6 hours of growth, the point at which it was predicted the cells would enter stationary phase (Fig. 4). This observation can be explained in part by the basal level of activation of the SOS response present in quiescent cells and its further induction in response to DNA damage (6). Gene products of the SOS response participate in DNA repair and this response is induced in quiescent stationary phase cells (6). For example, the *umuDC* genes, and the corresponding umuDC gene products, that are regulated in

conjunction with the SOS response have been shown to increase survival of cells in stationary phase by preventing entry into exponential phase until any DNA damage has been repaired (6). Another factor involved in the increased survival seen in stationary phase cells may be the increased synthesis of the  $\beta$ -clamp and RecF protein under the control of  $\sigma^S$ . Recent studies have revealed that when DNA replication is disrupted by UV exposure, RecF is involved in resuming the replication at DNA replication forks (8).

Figure 5 shows the relative frequency of survival of the cells grown on lactose and exposed to UV to the cells grown on glycerol and subjected to the same treatment. The first portion of the graph (to 2.5 hours) is relatively flat, indicating that the difference in survival between the cells grown on the two different types of media was constant. From 3 hours onward the graph became quite erratic. The format of the graph is such that it magnifies the error in the data, and from 3 hours onwards there were a relatively large number of plates that did not have a statistically accurate number of colonies due to errors in the dilutions. This led to some inaccuracy in figures 3 and 4, and this inaccuracy is magnified in figure 5.

In summary, there is decreasing survival from 0-3 hours as the *E. coli* are in exponential growth followed by increasing survival to UV exposure as the cells enter stationary phase, which indicates an upregulation of DNA repair and survival mechanisms.

### FUTURE EXPERIMENT

This experiment was intended to examine the efficiency and accuracy of DNA repair mechanisms in *E. coli* SMR4562 at different growth stages. To do this, it was initially planned to measure the difference in the mutation rates of *E. coli* SMR4562 at various growth stages by measuring the frequency of UV-induced Lac<sup>+</sup> reversions. However, the starting culture was mixed, containing already reverted Lac<sup>+</sup> bacteria. Despite this, it was possible to analyze the survival differences between bacteria at different growth phases. To specifically address the growth phase dependent DNA repair mechanisms aspect of this experiment, it would be desirable to use a Lac<sup>-</sup> culture that is ensured for purity, to carry out the experiment.

Furthermore, it would be interesting to investigate the degree of expression of some of the DNA repair genes, such as *recA*, *uspA* and *umuDC* in the context of the difference seen in survival of cells in stationary and exponential phase. This could be done using various molecular techniques, for example, a Western blot using antibodies specific for RecA, UspA and UmuDC. The level of expression of these genes could also be examined through reverse transcriptase-PCR (4).

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