

The Analysis of Mutagenesis Results of WP2 Bacteria By MMS and UV light

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Abstract: The WP2 assay, also known as the tryptophan reverse mutation assay, detects the production of additional revertants induced from tryptophan auxotrophs, indicating a positive mutagenic effect. This experiment aimed to investigate bacterial mutagenesis using the chemical methyl methanesulfonate (MMS) and ultraviolet (UV) light to assess their impact on cells. The experimental approach involved determining the appropriate *E. coli* concentration based on the OD value, applying MMS and UV mutagenesis treatments to each plate, estimating the number of viable cells through serial dilution, and setting up different control groups to calculate spontaneous mutations and assess the degree of mutation induction. The results indicate that both MMS and UV radiation have mutagenic effects on *E. coli* auxotrophs. However, these effects exhibit a non-linear relationship with MMS concentration. Additionally, a relatively short UV irradiation time induces noticeable mutagenesis, while excessively long exposure may lead to a reduction in bacterial colonies due to the damaging effects of prolonged UV exposure. The adverse cytotoxicity of MMS appears to obscure mutagenic effects in treatment plate assays.

1 Introduction

Mutagens are substances that can cause sudden or fundamental changes in an organism's genetic material, leading to genetic mutations or chromosome aberrations beyond natural levels [?]. The genetic toxicity test using *Escherichia coli* WP2 has been widely employed in assessing the safety of food, medicine, cosmetics, and the environment due to its high sensitivity to various mutagens [?]. *Escherichia coli* tryptophan auxotrophic strains, such as WP2 *trp*⁻, cannot synthesize tryptophan due to a mutation in the trpE65 allele, which contains an ochre stop codon (TAA) instead of a glutamine codon (CAA) [?].

This assay is valuable for mutagen screening because it allows only revertant bacteria to grow on medium lacking tryptophan. Spontaneous reversion mutations occur at a low frequency. In the presence of a mutagenic substance, auxotrophic bacteria may revert to prototrophic type through two main mechanisms. The first involves a mutation within the original ochre codon, changing TAA to glutamine (CAA), glutamic acid (GAA), leucine (TTA), serine (TCA). Alternatively, a second location mutation may occur involving an ochre suppressor mutation at anticodon sites in tRNA genes.

Prolonged UV exposure would kill too many bacteria to be detected, given UV light's disinfection function. However, defining what constitutes prolonged exposure is challenging [?] [?]. For instance, while 20 seconds of exposure may be suitable for inducing mutations, a 40-second period is not appropriate. The specific trends within the 20-second interval are not characterized. Therefore, additional experimental groups with smaller exposure intervals are required to comprehensively study UV mutagenesis [?] [?].

Therefore, in addition to spontaneously developed revertants, there will be additional revertants capable of growing and forming colonies on tryptophan-limited medium in the presence of a mutagen, indicating a positive result. The *E. coli* WP2 strain is a commonly used

genetic marker in radiation studies, DNA repair mechanism analysis, and mutagenesis research. In this experiment, we aim to investigate chemical mutagenesis using methyl methanesulfonate (MMS) and UV light mutagenesis using the WP2 assay with an *E. coli* *trp*⁻ strain under different conditions. Specific control groups are included for estimating viable cell counts and determining spontaneous mutation rates.

2 Material and Method

Table 1. Materials needed for this experiment

Cells Preparation	Explanation
<i>E. coli</i> <i>Trp</i> ⁻ strain	
Liquid growth medium	
PBS, pH 7.4	>25 mL
1.5 mL microcentrifuge tubes	1
Serial dilutions	
<i>E. coli</i> <i>Trp</i> ⁻ culture suspension (10 ⁸ cells/mL)	0.1mL
1.5 mL microcentrifuge tubes	6
PBS	5.5
Cell mutagenesis	
SA1 plate (0.25 µg/mL tryptophan)	2
SA2 plate (1 µg/mL tryptophan)	6
SA3 plate (0 µg/mL tryptophan)	2
NA plate (plenty of tryptophan)	2
<i>E. coli</i> <i>Trp</i> ⁺ culture suspension	0.1mL
<i>E. coli</i> <i>Trp</i> ⁻ culture suspension (10 ⁸ cells/mL)	1mL
<i>E. coli</i> <i>Trp</i> ⁻ culture suspension (10 ³ cells/mL)	0.1mL
<i>E. coli</i> <i>Trp</i> ⁻ culture suspension (10 ² cells/mL)	0.1mL
1% MMS	50µL
2% MMS	25µL
Sterile water	25µL
Sterile inoculating loop	1
Sterile beads	

2.1 Cells Preparation

Inoculate *Escherichia coli* into a liquid culture medium. After 2.5 hours, begin monitoring the optical density at 600 nm (OD600) of the liquid growth medium as a con-

trol, checking every 30 to 15 minutes until the value reaches 0.30-0.35, indicating a concentration of approximately 2×10^8 E. coli cells/mL in the mid-logarithmic phase. Subsequently, wash the bacteria by removing the growth medium and centrifuging at 10,000 rpm for 10 minutes. Following removal of the supernatant, resuspend the pellet in 15-20 mL of phosphate-buffered saline (PBS) at pH 7.4. Centrifuge again at 10,000 rpm for 10 minutes, then resuspend the pellet in 4 mL of PBS and use PBS as a blank to check the OD600. Distribute cells into multiple 1.5 mL microcentrifuge tubes labeled "WP2 *trp*⁻".

2.2 Serial Dilutions

Six sterile tubes were labeled from 10^{-1} to 10^{-6} and each was filled with 0.9 mL of sterile phosphate-buffered saline (PBS). Subsequently, 0.1 mL of the bacterial suspension was transferred to the 10^{-1} tube and mixed thoroughly by vortexing. This process was repeated for each subsequent dilution, with 0.1 mL of the previous dilution being transferred to the next tube. The dilution process was continued until all tubes were completed. The 10^{-5} and 10^{-6} diluted cultures were then ready for culture plating.

2.3 Cell Mutagenesis

To provide a visual representation of the experimental setup, Table 2 was used to outline each plate's composition. Plate 1 was divided into two halves, with one half streaked with E. coli *trp*⁺ cells using a sterile inoculating loop, and the other half streaked with E. coli WP2 *trp*⁻ cells. Plates 2 to 4 received 0.1 mL of undiluted bacterial culture each, plated onto corresponding SA plates. NA plates 5 and 6 were plated with diluted bacterial suspensions (10^{-5} and 10^{-6} , respectively). Plates 7 to 9 had 0.1 mL of undiluted bacterial suspension spread onto each plate, followed by the placement of a sterile paper disc at the center of each plate using sterile forceps. Subsequently, 25 μ L of 1% MMS, 2% MMS, and sterile water, respectively, were added onto the disc. A microcentrifuge tube containing 0.5 mL of WP2 bacterial culture and 5 μ L of 1% v/v MMS was incubated at 37°C for 30 minutes. After incubation, 0.1 mL of the treated sample was plated onto SA plate 10. The same treatment was applied to plates 11 and 12, with the exception of the exposure period to UV light. After spreading the cell culture and allowing it to dry, plates 11 and 12 were exposed to UV light without their lids, irradiated with $10 J/m^2$ of UV for 20 seconds and 40 seconds, respectively. All plates were then placed in baskets with their lids and incubated overnight at 37°C. Colonies were observed the following day.

Table 2. Outline of the mutagenesis for each plate

Plate Number	Cell dilution to be plated	Plate type	Tryptophan concentration(μ g/mL)	Treatment
1	10^0	SA3	0	<i>Trp</i> ⁺ strain and <i>Trp</i> ⁻ strain
2	10^0	SA3	0	None
3	10^0	SA2	1	None
4	10^0	SA1	0.25	None
5	10^{-5}	NA	Non-limiting	None
6	10^{-6}	NA	Non-limiting	None
7	10^0	SA2	1	Spot test with 1% MMS
8	10^0	SA2	1	Spot test with 1% MMS
9	10^0	SA2	1	Spot test control (water)
10	10^0	SA1	0.25	Treat with 1% MMS then plate
11	10^0	SA2	1	Irradiate with UV light for 20 sec
12	10^0	SA2	1	Irradiate with UV light for 40 sec

3 Results and Analysis

The results of the bacterial mutagenesis experiment can be determined by the corresponding number of individual colonies of Escherichia coli. A summary is presented in Table 3 for comparison. Plates 1-4, as represented in Figure 1, were designed for estimating spontaneous revertant mutations, with plates 2-4 serving this purpose, while plate 1 was divided into positive and negative controls. In plate 1, half of normal *trp*⁺ strains have dense colonies, while the other half of the *trp*⁻ strain showed no signs of bacterial growth. The negative control was consistent with expectations, as the tryptophan-deficient cells cannot grow without tryptophan supplementation unless some of them undergo spontaneous revertant mutations to regain the ability to synthesize tryptophan. For plates 2-4, only a few single colonies were observed after overnight incubation, which can be used to estimate the

frequency of spontaneous revertant mutations. The numbers of single colonies on plates 2-4 were 0, 2, and 10, respectively. The only difference between these three untreated plates was the concentration of tryptophan provided, with concentrations of 0, 1, and 0.25 μ g/mL for plates 2-4, respectively. The lack of growth of colonies on plate 2, which lacked tryptophan in the medium, was an expected result. Theoretically, the number of colonies produced on plate 3, which had four times the tryptophan concentration of plate 4, should be greater than on plate 4. However, due to the absence of replicates or triplicates to avoid unexpected circumstances, and the lack of a significant difference in colony numbers, no definitive conclusion can be drawn from a simple comparison.

Table 3. Count of bacterial colonies on each plate

Plates	1	2	3	4	5	6	7	8	9	10	11	12
colonies	/	0	2	16	4	0	32	59	5	2	7	3

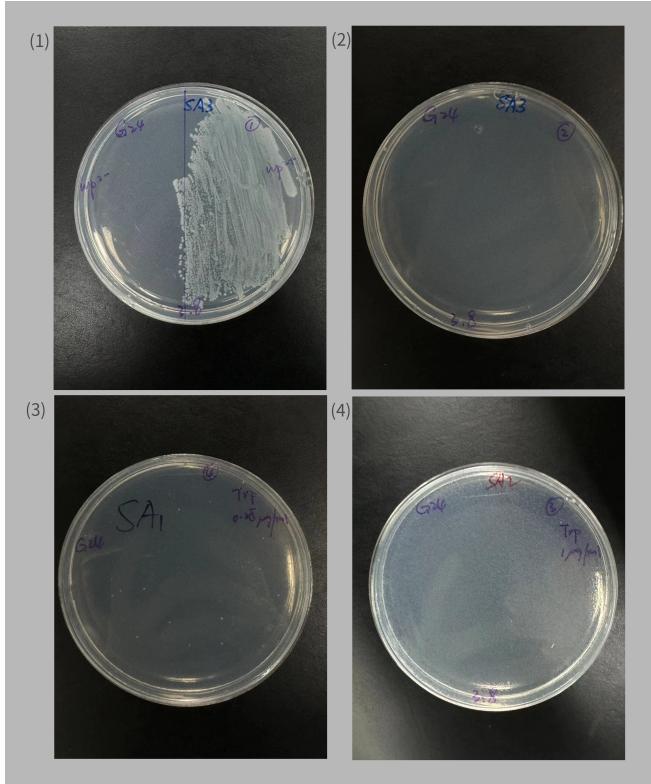


Figure 1: plate 1-4

The results of plates 5 and 6 are shown in Figure 2. Both plates contained nutrient agar medium with sufficient tryptophan, expected to support the normal growth of tryptophan auxotrophic cells. These plates were designed with consecutive dilution factors of 10^5 and 10^6 to provide an appropriate range for counting colony-forming units (CFUs). However, as shown in Figure 2, plates 5 and 6 exhibited almost no visible colonies, contrary to expectations, making it impossible to calculate the colony concentration further. This discrepancy suggests that the absence of colonies was likely due to the omission of tryptophan in the agar medium preparation.

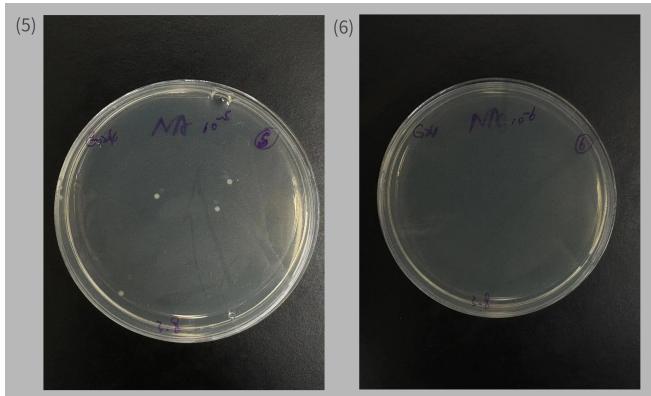


Figure 2: plate 5 and plate 6

Figure 3 illustrates the results of plates 7-10. The

third group underwent chemical mutagenesis using MMS to assess mutagenicity in *Escherichia coli*. Two types of screening assays were conducted. For the spot test groups on plates 7-9, the water control (plate 9) was effective, as only 5 colonies were observed on the plate, representing spontaneous revertants from the spot test. Compared to spontaneous mutations, it is evident that the mutation frequency induced by MMS was much higher than the control group, with 32 colonies on plate 7 and 59 colonies on plate 8. Colonies in plates 7 and 8 were unevenly distributed. A clean circular inhibition zone was observed near the center spot, indicating the absence of colonies [?]. Most colonies were located near the spot but did not invade it, and a few colonies were found at the edges of the plates. This phenomenon may indicate that revertants near the center may be dead, making the data less reliable. The number of revertants on plates treated with MMS was 15-30 times higher than that of spontaneous revertants. The difference in the number of revertants between 1% and 2% MMS solutions was approximately 2-fold, indicating a simple linear relationship between MMS concentration and WP2 revertant frequency.

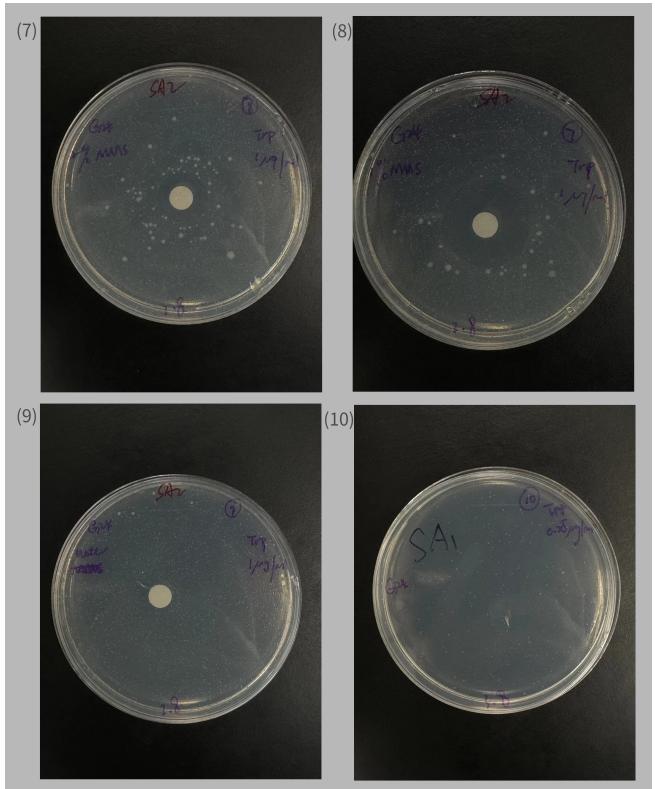


Figure 3: plate 7-10

Regarding the plate incorporation test, plate 10 results showed only 2 colonies, similar to the results of spontaneous mutations. Theoretically, the plate incorporation test is more sensitive than the spot test because it allows the bacteria to be fully treated with the mutagen in the liquid suspension. However, the significantly lower number of colonies on plate 10 compared to plates 7 and 8 can be explained by the cytotoxic effects of MMS. When MMS induces bacterial mutations, it simultaneously kills many bacteria. Many induced revertants were likely killed

before they could proliferate to form colonies [?]. Finally, plates 11 and 12 (representing Group 4, used to explore the effects of UV light) yielded 7 and 3 colonies, respectively (Figure 4). It can be inferred that exposure to UV light for 20 seconds resulted in more viable revertants compared to exposure for 40 seconds.

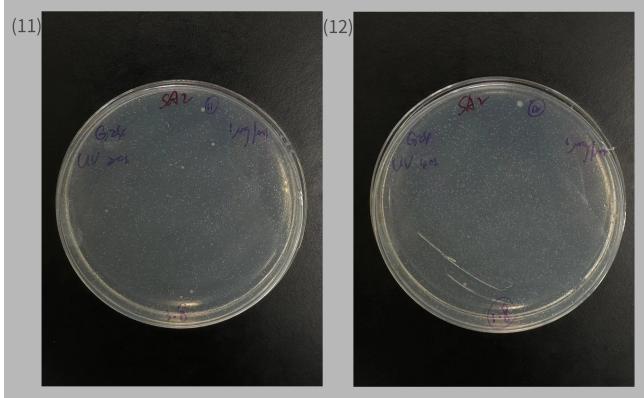


Figure 4: plate 11 and plate 12

Due to the unexpected outcomes of plates 5 and 6, it was not possible to calculate the expected total number of viable cells. Referencing the data from Group 8 and Group 23, Table 4 was derived. It was observed that plates 5 and 6 in these two groups exhibited abundant colony growth. Using the data from Group 8, a series of calculations including CFUs were performed. The colony count on plate 6 was used to infer the assumed viable cells, with the countable range of colonies being between 30 and 300. Although the viable cells were assumed to have a value of 5.6×10^8 cfu/mL, the expected viable cells per treatment plate were 5.6×10^7 due to the 0.1 mL volume of culture material added to each plate. Plate 2 was used to estimate the spontaneous mutation frequency, which was calculated as 1.79×10^{-8} . This was because all observed single colonies on plate 2 originated from the *trp⁺* revertants in the bacterial culture before plating, whereas the corresponding plates for the other two groups were developed from both the original revertants and newly emerged revertants during the initial cell division phase.

Table 4. Statistics on the number of colonies in groups 8 and 23

Plates	1	2	3	4	5	6	7	8	9	10	11	12
G8	/	1	3	5	550	56	44	91	6	6	21	6
G23	/	2	12	3	680	142	48	70	2	5	3	48

4 Discussion and Conclusion

This study aimed to investigate the mutagenic effects of MMS and UV light on WP2 *trp*. The findings suggest that both MMS and UV light are mutagenic to *E. coli* auxotrophs, with mutation rates increasing with MMS concentration, albeit not in a linear fashion. Shorter UV light exposure periods favored reverse mutagenesis, while longer exposures resulted in fewer colonies. However, the mutagenic impact of MMS in the treat-and-plate test appeared to be masked by its cytotoxicity.

The logarithmic relationship between MMS dose and mutation frequency showed a positive linear correlation, consistent with previous research. For instance, the plate treated with 2% MMS exhibited approximately twice as many revertant colonies as the plate treated with 1% MMS, suggesting a possible positive correlation. However, due to the limited number of test groups with varying concentrations, a detailed understanding of this relationship was not achievable.

The overall conclusions regarding the mutagenic effects of both MMS and UV light are compelling, considering the differences in scales between spontaneous and induced mutation frequencies. Molecular rationale further supports these conclusions. MMS induces mutations by methylating purines, resulting in the formation of toxic non-canonicals that are excised to form apurinic sites for further base transversion. UV light induces mutations by forming base dimers and through the function of error-prone polymerase, supporting its mutagenic effects at the molecular level [?].

Prolonged UV exposure would kill too many bacteria to be detected, given UV light's disinfection function. However, defining what constitutes prolonged exposure is challenging. For instance, while 20 seconds of exposure may be suitable for inducing mutations, a 40-second period is not appropriate. The specific trends within the 20-second interval are not characterized. Therefore, additional experimental groups with smaller exposure intervals are required to comprehensively study UV mutagenesis.

The unexpected results of plate 10 can be attributed to the cytotoxicity of MMS, which decreases the survival ratio of WP2 with increasing MMS dose. The clear inhibition zone observed in the spot test plate further supports the toxicity of MMS [?].

1. Count the number of colonies on each of the plates (plates 1-12).

(table4.G8)

Plate 1: many	Plate 2: 1	Plate 3: 3	Plate 4: 5	Plate 5: 550	Plate 6: 56
Plate 7: 44	Plate 8: 91	Plate 9: 6	Plate 10: 6	Plate 11: 21	Plate 12: 6

2. Using the count from Plates 5, and 6, estimate the number of cells plated on each of the plates.

Using this figure, you should be able to estimate the spontaneous mutation frequency. How do you calculate the spontaneous mutation frequency?

What is your estimate of the spontaneous mutation frequency?

We chose plates with 30-300 colonies, as this range is considered statistically significant. Therefore, we selected plate 6 to estimate the number of cells plated on each plate. Plate 6 had a dilution factor of 10^6 , with 0.1 ml of undiluted WP2 cell suspension used for each plate. So, the number of cells plated on each of the plates = 5.6×10^7

First, determine the number of spontaneous mutation colonies. Then, determine the number of viable cells on each of the plates. In the end, calculate the Spontaneous mutation frequency using the equation below:

$$\text{Spontaneous mutation frequency: } \frac{\text{colonies on the plate 2}}{\text{The number of viable cells on the plates}}$$

$$\text{Spontaneous mutation frequency} = 1 / 5.6 \times 10^7 = 1.79 \times 10^{-8}$$

3. Similarly, estimate the apparent spontaneous mutation frequency on the SA2 and SA1 plates (Plates 3 and 4). Why are these only apparent frequencies, and why do they differ from the true frequency estimated from SA3, and from each other?

Apparent spontaneous mutation frequency of plate 3

$$\frac{\text{colonies on the plate 3}}{\text{The number of cells plated on the plates}} = 3 / 5.6 \times 10^7 = 5.36 \times 10^{-8}$$

Apparent spontaneous mutation frequency of plate 4

$$\frac{\text{colonies on the plate 4}}{\text{The number of cells plated on the plates}} = 5 / 5.6 \times 10^7 = 8.93 \times 10^{-8}$$

In plate 1, both plates were supplemented with tryptophan, allowing the WP2 trp⁻ strain to multiply and divide after plating. During this propagation, spontaneous mutations occurred, leading to the formation of WP2 trp⁺ revertants. Therefore, the observed colonies in the medium were a mixture of original WP2 trp⁺ revertants in the cell suspension and

newly generated WP2 trp⁺ revertants after culture.

In contrast, no tryptophan was added to plate 2. Consequently, the WP2 trp⁻ strain in the cell suspension could not reproduce after plating. Thus, the colonies observed in plate 2 consisted solely of original WP2 trp⁺ revertants, which had undergone spontaneous mutation before culture plating. When calculating the spontaneous frequency, it was assumed that the total number of cells in each medium was the same. However, due to the addition of tryptophan, the quantity of WP2 trp⁺ revertants in plates 3 and 4 was higher than in plate 2, leading to a different apparent frequency in plates 3 and 4 compared to plate 2.

The discrepancy in apparent frequency between plates 3 and 4 was attributable to the varying amounts of tryptophan added to each plate, resulting in the production of different quantities of WP2 trp⁺ revertants during the propagation process of the WP2 trp⁻ strain. This discrepancy affected the apparent frequency observed in the two plates.

4. Estimate the mutation frequency induced by MMS. You should compare the mutation frequency with one of the spontaneous frequencies you calculated. From which plate should you determine the spontaneous frequency?

Mutation frequency induced by MMS

colonies on the treated plate – colonies on the control plate

The number of viable cells on the plates

$$\text{Plate 7: } \frac{\text{colonies on the plate 7} - \text{colonies on the plate 9}}{\text{The number of viable cells on the plates}} = \frac{(44-6)}{5.6 \times 10^7} = 6.79 \times 10^{-7}$$

$$\text{Plate 8: } \frac{\text{colonies on the plate 8} - \text{colonies on the plate 9}}{\text{The number of viable cells on the plates}} = \frac{(91-6)}{5.6 \times 10^7} = 1.52 \times 10^{-6}$$

$$\text{Plate 10: } \frac{\text{colonies on the plate 10} - \text{colonies on the plate 4}}{\text{The number of viable cells on the plates}} = \frac{(6-5)}{5.6 \times 10^7} = 1.79 \times 10^{-8}$$

The mutagenicity of plates 7 and 8 should be compared to the spontaneous mutation frequency in plate 9. A limited amount of tryptophan in these plates allowed only a subset of cells to divide and produce WP2 trp⁺ revertants. The experimental conditions were controlled, ensuring that all three plates underwent the same experimental method (spot test) and received the same amount of tryptophan. In plates 9, 7, and 8, WP2 trp⁺ revertants were produced spontaneously, with the existing revertants assumed to be the same. Hence, the mutation frequency induced by MMS should be compared with that of plate 9. Plate 10 should be compared to plate 4, as they share the same tryptophan

concentration and plating method.

5. Estimate the mutation frequency induced by the UV dose of 10 J/m². Compare this frequency to that induced by MMS. Which treatment shows the highest induced mutation frequency?

Is it fair to directly compare the two frequencies?

Mutation frequency induced by UV

$$\frac{\text{colonies on the treated plate} - \text{colonies on the control plate}}{\text{The number of viable cells on the plates}}$$

$$\text{Plate 11: } \frac{\text{colonies on the plate 11} - \text{colonies on the plate 3}}{\text{The number of viable cells on the plates}} = (313-3)/5.6 \times 10^7 = 5.54 \times 10^{-6}$$

$$\text{Plate 12: } \frac{\text{colonies on the plate 12} - \text{colonies on the plate 3}}{\text{The number of viable cells on the plates}} = (7-3)/5.6 \times 10^7 = 7.14 \times 10^{-8}$$

20 seconds of UV treatment with a dose of 10 J/m² shows the highest induced mutation frequency

No, it wouldn't be appropriate to directly compare the mutation frequencies between the two treatments. MMS and UV light are distinct in their mechanisms of action—one is chemical, and the other is based on light exposure. While it might be tempting to compare the highest frequency observed in each treatment, it's important to note that the 40-second UV exposure resulted in fewer colonies than plates 7 and 8, despite potentially having a higher mutation frequency. Additionally, plate 10's results are challenging to compare, as it represents a different test setup. Overall, finding a suitable criterion for direct comparison may be challenging in this context.

6. Record the appearance of the MMS spot plates.

Do you think it is valid to count the colonies on this dish? Provide explanations for your answer.

What can you say about the relationship between MMS concentration and the number of mutants?

Clear zones formed around the disc where MMS was dripped inhibited bacterial growth, with no E. coli colonies present within these zones. Most WP2 trp+ revertants grew

scattered around the clear areas, while a few were distributed towards the plate margin. The number of WP2 trp+ revertants in the plate supplemented with 1% MMS was fewer than that in the plate with 2% MMS. In comparison, the water control group showed no clear zones, with only several colonies appearing in a random distribution.

However, this method may be invalid. The observed number of colonies on the plate may not accurately reflect the actual number of colonies obtained through MMS-induced mutation under normal cell conditions. Due to its cytotoxic nature, MMS can kill some E. coli revertants, leading to the formation of clear zones around the disc. While MMS can increase mutation frequency, the partial death of E. coli may decrease the number of spontaneous mutations. Additionally, the location and sizes of colonies are important considerations. As the MMS concentration varies from the center to the margin of the plate, the colony sizes may reflect their viabilities. These factors should be taken into account when studying MMS mutagenesis. Therefore, counting colonies on this plate may not be a valid method.

Furthermore, there appears to be a positive correlation between MMS concentration and the number of mutants, particularly between 1% and 2% MMS concentration (with a linear correlation observed in their log-scale values with a slope of 1.65). However, outside this concentration range, the relationship is uncertain.

7. You are provided with compound Z which is suspected to cause cancer in humans. You test the compound using the WP2 assay (both the spot test and liquid culture test) and find it to be negative (no increase in revertants compared to the control). Another laboratory finds Z induces cancer in rodents.

Can you be sure whether compound Z is a mutagen or non-mutagen?

How might you redesign your experiment to determine whether Z is mutagenic or not?

The WP2 assay is a common method used to assess the mutagenicity of chemicals. However, its limitation lies in its inability to determine whether a single chemical is a mutagen. A pro-mutagen is a chemical that, on its own, cannot induce mutation. Still, with the presence of metabolic activity to activate the pro-mutagen, it can be converted into a mutagen, potentially leading to cancer.

Compound Z is a candidate for being a mutagen. Despite not showing mutagenic effects in the WP2 assay, it has been found to cause cancer in rodents. This discrepancy could be due to metabolic activity.

However, definitive conclusions about compound Z as a pro-mutagen are challenging due to other potential factors affecting the results. To test whether compound Z is mutagenic,

three sets of experimental samples are required. The first set of cells would serve as a control and remain untreated. The second set would be treated with compound Z, incubated, and then observed for normal cell growth. The third set would be treated with compound Z and rat liver extract containing metabolic enzymes.

If compound Z is a mutagen (pro-mutagen), cells in the first and second groups would grow normally, while the third group would exhibit malignant transformation, indicating that compound Z was converted into a mutagen by the rodent's metabolic enzymes, leading to cancer. If all three groups of cells grow normally, compound Z is not a mutagen.

8. Suggest 3 ways in which the sensitivity of the WP2 assay might be increased.

To enhance the sensitivity of the WP2 assay, several strategies can be employed. Firstly, using more sensitive strains such as WP2 uvrA or strains with plasmid pKM101 can improve sensitivity to mutagens like MMS. Additionally, altering cell permeability can make cells more predisposed to mutagens, thus increasing assay sensitivity.

Furthermore, to avoid the adverse effects of high MMS doses, it's crucial to determine appropriate concentration ranges in advance. This can be achieved by setting up more detailed concentration steps for the chemical reagents used in the experiment. Similarly, dividing UV irradiation time into smaller intervals can help identify the most appropriate range of irradiation times.

Maintaining a sterile environment and ensuring proper disinfection of laboratory equipment are essential to prevent contamination and maintain the sensitivity of the assay. Additionally, experiments should be repeated to ensure the reliability of results. Conclusions should be based on the results of at least two independent experiments to improve the sensitivity of the assay.

Lastly, utilizing more suitable software and instruments for result analysis can enhance the sensitivity of the assay. While the spot test may be less sensitive than the treat-and-plate test, choosing the latter method can improve sensitivity if higher sensitivity is required.



Department of Biological Sciences

BIO204 - JOINT LAB REPORT

Name of Practical: Genotyping and Population Genetic Analysis

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Student 3	Yimeng.Yuan	2146430		
Student 4	Haozhan.Yuan	2036887		
	Student 1	Student 2	Student 3	Student 4
Peer Mark by Student 1*		100	100	100
Peer Mark by Student 2*	100		100	100
Peer Mark by Student 3*	100	100		100
Average Peer Mark*	100	100	100	
Relative Peer Contribution*	100%	100%	100%	100%
Group Lab Report Mark				
Individual Lab Report Mark				