Prelab Exercise

Read the lab manual, websites, and primary literature online and answer the following questions on a separate sheet of paper. This is part of your lab grade. You will use your research for the intro of your PP presentation. This WILL BE CHECKED IN CLASS DURING THE PRELAB QUIZ!

1. What is your compound?

2. Make sure your compound is FULLLY SOLUBLE IN AQUEOUS SOLUTION. Mix it with some water in a disposable cup. If it doesn't fully go into solution, go back to Step 1.

3. What is your hypothesis?

4. What research did you do to develop your hypothesis? Cite your sources.

5. How much of your compound are you proposing to use in the dose/response curve? What is your rationale for this procedure?

Using Yeast to Test Household Compounds for Mutagenic Activity

Objectives:
In doing this exercise you will:
1. Formulate a hypothesis about the mutagenic potential of a household chemical that you bring from home.
2. Test your hypothesis using a protocol from primary literature.
3. Analyze your data using Excel graphing, comparing to positive and negative controls.
4. Present your hypothesis, data, and analysis in a PowerPoint presentation.

Introduction:
Read Chapter 16 in your book. Read the paper by F.K. Zimmerman et al., the paper by F.K. Zimmerman, and also the websites posted in Blackboard on yeast.

The strain you are going to work with has been genetically engineered to have differing phenotypes depending on DNA mutations (and subsequent DNA repair) that have occurred.

Mutation	Phenotypes on Different Media		
	Complete	Lacking Tryptophan	Lacking Isoleucine
None (i.e. starting strain)	White	No growth	No growth
Mitotic Crossing Over (recombinational repair)	Red and pink sectored colonies	No growth	No growth
Mitotic Gene Conversion (gap repair synthesis followed by mismatch repair)	White	Growth	No growth
Reverse (point) Mutation	White	No growth	Growth

When DNA is mutated, depending on the mutation, there are many ways in which eukaryotes can fix the DNA lesions. These include direct repair, base excision repair, nucleotide excision repair, mismatch repair, and recombinational repair. General Genetic textbooks cover these in detail (for example Chapters 16 and 17 in Brooker) so I won't go into them. This yeast strain has different phenotypes depending on the DNA mutations and the ensuing DNA repair mechanism that has occurred.

You will be using this yeast strain, D7, and chemicals you bring from home along with a protocol you devise to test these chemicals for their mutagenic potential in a eukaryote.

Pre Lab Procedure:

1. Think about what chemicals from your home might indeed be mutagens. **WHATEVER YOU BRING MUST BE FULLY SOLUBLE IN AQUEOUS SOLUTION. LIQUIDS ARE BETTER THAN SOLIDS, BUT THE LIQUID NEEDS TO BE FULLY SOLUBLE IN AQUEOUS SOLUTION (i.e. nothing organic, no oils, etc). IF YOU BRING IN A SOLID, IT MUST BE FULLY SOLUBLE IN AQUEOUS SOLUTION. NOT JUST A SUSPENSION, BUT SOLUBLE.** If you are not sure if it's soluble, you can test it at home by checking to see if you can dissolve it in water. Perform research on the chemicals, and find one that indeed could be a **MUTAGEN** (not just cytotoxic but a mutagen). Bring this chemical into lab with your research and justification. A justification can be an article from a newspaper, magazine or reputable online news source (like CNN.com etc), primary literature, or other reputable source of information.
2. Propose a hypothesis about your household compound.
3. Read the papers and websites posted online to understand what *Saccharomyces cerevisiae* is and what the test is that we are going to be performing.
4. Design a series of treatments of the D7 cells that will answer the question: is this chemical a mutagen or not? Use a dose/response relationship (described below).

Things to think about:

1. Just because a compound is toxic does not mean it is a mutagen. Toxicity and mutagenicity are two different things. Bring a suspected mutagen.
2. Don't bring a compound that kills cells. For example, something that contains bleach (sodium hypochlorite) alcohol (any type including cetyl alcohol, isopropanol, and ethanol), or detergents (soaps) will kill the cells. So, no hand soap, body soap, or shampoos, for example. No drain cleaner, shower cleaner, or window cleaner.
3. You will want a positive control and a negative control.
   a. A positive control is something that we already know causes mutations. This allows us to ensure our assay is working correctly.
   b. A negative control is something that will not cause mutations. We will use water. We will compare all results to the negative control to see if the rate of mutation of your treatments is greater than that of the negative control. For more information about negative controls see page 450 in your book.
4. You will want to use different dilutions of your suspected mutagen. This is called a **dose/response relationship**. This shows that your compound is indeed a mutagen. For example if you use 10 μl, 20 μl, 30 μl, and 40 μl of your compound, then if it is indeed a mutagen, you would hypothesize that there will be two times as many colonies on the 20 μl as on the 10 μl, three times on the 30 μl plate, and 4 times on the 40 μl plate. If all of the plates show the same or similar number of colonies, then your compound is probably not a mutagen, because increasing amounts of your compound should cause increasing amounts of mutagenesis.
5. If your suspected mutagen is a solid, how do you want to resuspend it in a liquid before using it for treatment? You will need a solid that can fully dissolve in aqueous solution forming a **SOLUTION NOT A SUSPENSION.**
6. What will the negative control tell you? What will the positive control tell you? What will each of your differing treatments tell you?

Lab Procedure:

1. You will have your hypotheses and procedure checked by your instructor before you are given yeast cells to use.
2. You will be given yeast cells in 0.1 M potassium phosphate pH 7. This is a stationary phase culture that has been diluted 1/100, so it has about 1 X 10^5 cells per ml.
3. Use 300 μl of cells per treatment.
3. You will have an unlimited amount of 0.1M potassium phosphate buffer to use.
4. You will treat your yeast cells with your suspected mutagen according to the protocol you designed; the instructor will perform the positive and negative controls.
5. We will all do treatments of 30 minutes, so that the time of treatment is not a factor.
6. EACH SEPARATE TREATMENT GETS SPLIT INTO THREE EQUAL ALIQUOTS (parts) of 100 μl each. Each aliquot gets plated onto one plate so that each treatment is put on all three plates in equal amounts:
   YPD
   Media lacking tryptophan
   Media lacking isoleucine

   So for example, if you treated your cells with 10 μl, 20 μl, 30 μl, and 40 μl of your suspected mutagen, you will have 12 total plates (4 different treatments multiplied by three plates for each treatment).
7. Each plate incubates at 28°C for a week. You will check phenotypes next lab period. If you have a digital camera you may bring it in to take pictures of your plates for your presentation. The library has digital cameras for checkout as well. We will not be providing digital cameras for you to use.

Analysis:

8. You will check phenotypes on each plate for each treatment. You will count your colonies using a sharpie to place a dot on the underside of the Petri dish to indicate a counted colony (so you don't count it again). Your instructor can show you how.
   a. On the YPD plate, you expect to see it covered with cells. This is called a **lawn**. You will be looking for red or pink colonies in a sea of white. If your YPD plate is not covered with a lawn (compare to the negative control), then your compound killed a lot of the cells. How is cell killing going to affect your analysis?
   b. On the plates lacking tryptophan or isoleucine, you should expect to see some white (the cells you originally plated). You will then be looking for colonies standing out above the white smear.
9. You will analyze your data using the graphing function in Excel.
   a. You will graph the data from the negative control and the positive control.
   b. Then, on a separate graph, you will put colony number on the Y axis and concentration on the X axis, for each of your concentrations.
   c. Compare the graph of the positive and negative controls with the graph of your compound. Is there a trend on the graph with the positive control suggestive of an effect caused by the mutagen? How does the graph of your compound compare to the controls? Is there a trend to indicate that your compound might be a mutagen?

d. If your suspected mutagen is toxic, as indicated by less colonies on any or all of your YPD plates than the negative control, you will have to analyze your data a second time. You will make more Excel graphs.
   i. On one graph, you plot concentration on the X axis and percentage of red or pink cells for each concentration on the Y axis. Percentage of red or pink cells is calculated as follows:

$$\frac{\text{number of red or pink colonies on the YPD plate}}{\text{total number of colonies on the YPD plate}} \times 100$$

   ii. Then you need to analyze your data from the other plates, using concentration on the X axis and this equation for the Y axis

$$\frac{\text{Number of colonies on the synthetic plate (i.e. lacking tryptophan or isoleucine)}}{\text{Total number of colonies from the corresponding YPD plate (of the same concentration of suspected mutagen)}} \times 100$$

   This number gives you an idea of how many colonies there are RELATIVE TO HOW MANY LIVE CELLS THERE WERE, since your suspected mutagen killed some of them.

   iii. What do your two new graphs tell you about your suspected mutagen? Is there now a trend indicating that your compound is a mutagen? How did you analyze your graphs to determine if there is a trend?

Presentation:

10. After determining your phenotypes, you will prepare a PowerPoint presentation that you will turn in to the instructor using the following guidelines:

Title: Generate a title such as "I determined that ?? is a mutagen!", not a generic title such as "Determination of Mutagenicity in Yeast Cells." This should be one slide long.

Introduction: Do not describe the cells, we all know what they are. Instead describe the chemical you brought in and why you thought it might be a mutagen. Include the research you did about your suspected mutagen here. What were your hypotheses and why? This should be 2-3 slides long.

Materials and Methods: Describe the controls you used and dilutions of your compounds. If you brought in a solid and placed it into solution, what concentration(s) did you use? Why did you do what you did? This should be 1-3 slides long.

Results: What did you see? You can put this in table form or in picture form, if you took digital pictures, or both. Also include your graphs. This should be 3-5 pages long

Discussion: Place your analysis here. What does this mean? Did you prove or disprove your hypotheses? If you disproved your hypotheses, what happened? Include what **future research** you would perform to **repeat your experiment** and show that your data is **relevant and significant**. Does your research have an application to our diet? They way we clean house?

What we feed our cats? How we treat cats for fleas? How we dispose of batteries? Etc Add anything else relevant here, but no opinions!! This should be at least 4 slides long.

References: Cite all references you used. This should be at least 1 page long. This should have at least the following references:
The reference(s) you used to decide which compound to bring in
The websites on yeast you read
This handout
The two primary literature sources you read
Your textbook

But may include more if you performed more research to prepare your presentation

Your PP presentation should be at least 12 slides long (including the Title and References slide). **It should be turned in as a hard copy with one slide per page. No electronic versions will be accepted. Make sure your PowerPoint**

Aspartame in Sweeteners Found to be Carcinogenic

Introduction

- The household product I brought in was Equal sweetener.
- The main ingredient in Equal I will be testing for mutagenic characteristics is Aspartame

Introduction

- Aspartame is a man made compound that is used as a sweetener in numerous different food products
- Aspartame was developed in 1965 by James Schlatter while he was trying to produce and anti-ulcer medication
- There has been an ongoing debate on whether Aspartame has mutagenic potential

Introduction

- “Early animal study revealing an exceedingly high incidence of brain tumors in aspartame-fed rats compared to no brain tumors in concurrent controls, the recent finding that the aspartame molecule has mutagenic potential, and the close temporal association” (Olney et al. 1996)
- “Higher levels of *aspartame* intake were not associated with the risk of overall hematopoietic *cancer*, giloma, or their subtypes in both men and women” (Lim et al. 2006)

Introduction

- With the debate on whether or not Aspartame is carcinogenic or not is still going on further testing in this lab may be beneficial.
- My hypothesis was that aspartame was mutagenic and would yield growth on the plates.
- The hypothesis will be test by growing yeast treated with various concentrations of aspartame on growth mediums.

Materials and Methods

- Three different aliquots were set up for the experiment, a YPD plate, the control, a plate lacking tryptophan, and a plate lacking isoleucine each aliquot had 4 plates.
- The test solution was prepared by combining Equal solution with a Potassium phosphate buffer until the solution was completely saturated and then 4 test solutions were made. 300 micro liters of cells were added to each test-tube.
- Different amount of the test solution where then placed into test tubes and treated in a hot water bath for 30 minutes.
- Each test solution was then plated using quantities of 100µl, 200µl, 300µl and 400µl.
- The cells were plated using a class hockey stick that was sterilized after each use via ethanol and a Bunsen burner. The plates were ethanol left to incubate for 7 days at room temperature
- The plates were then observed for any sign of mutagenic activity.

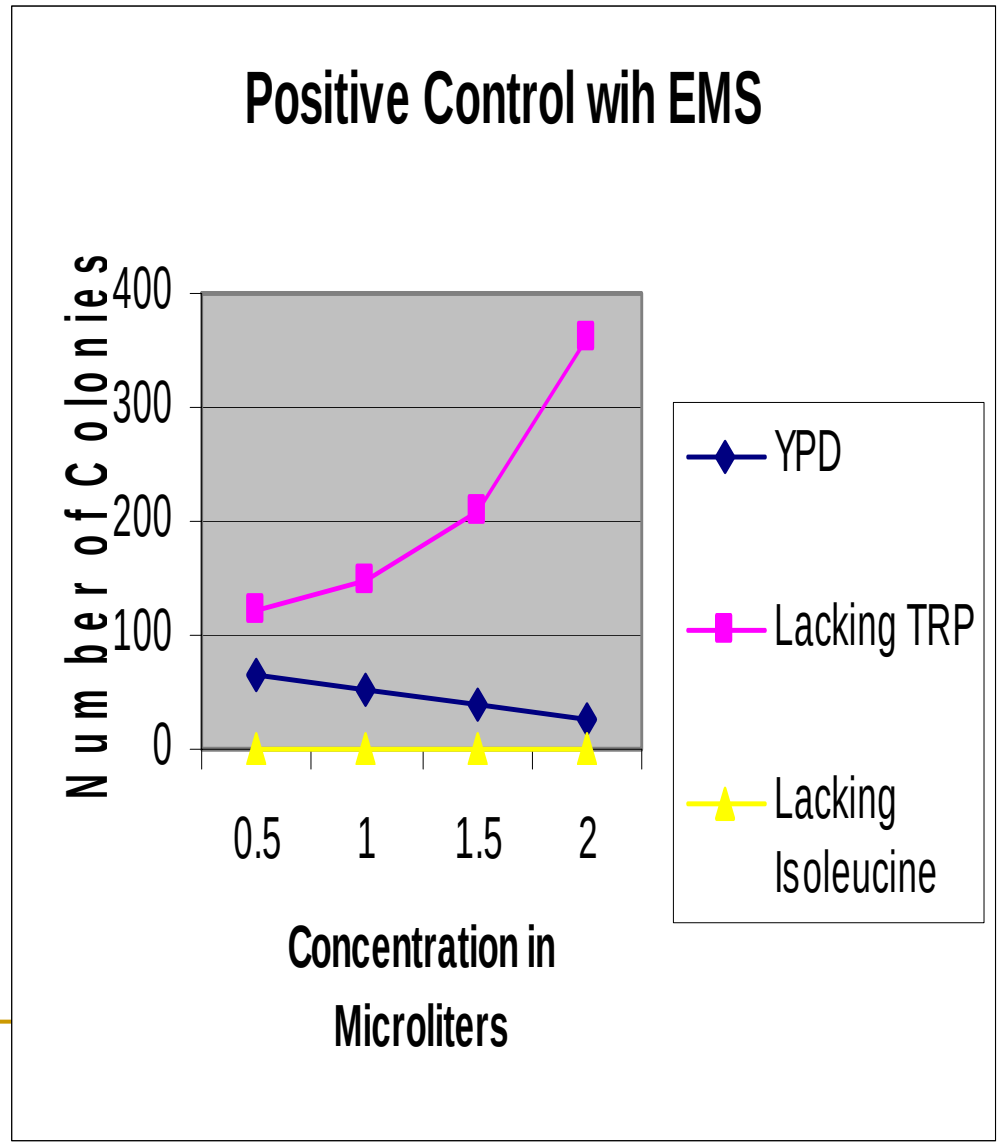
Results

- The results of this experiment showed various amounts of growth on the mediums.
- The controls used in this experiment were water, negative control, and Ethyl Methane Sulfate as the positive control.
- The following tables show the amount of concentrations used and the number of colonies that were formed.

Results

Positive control with EMS

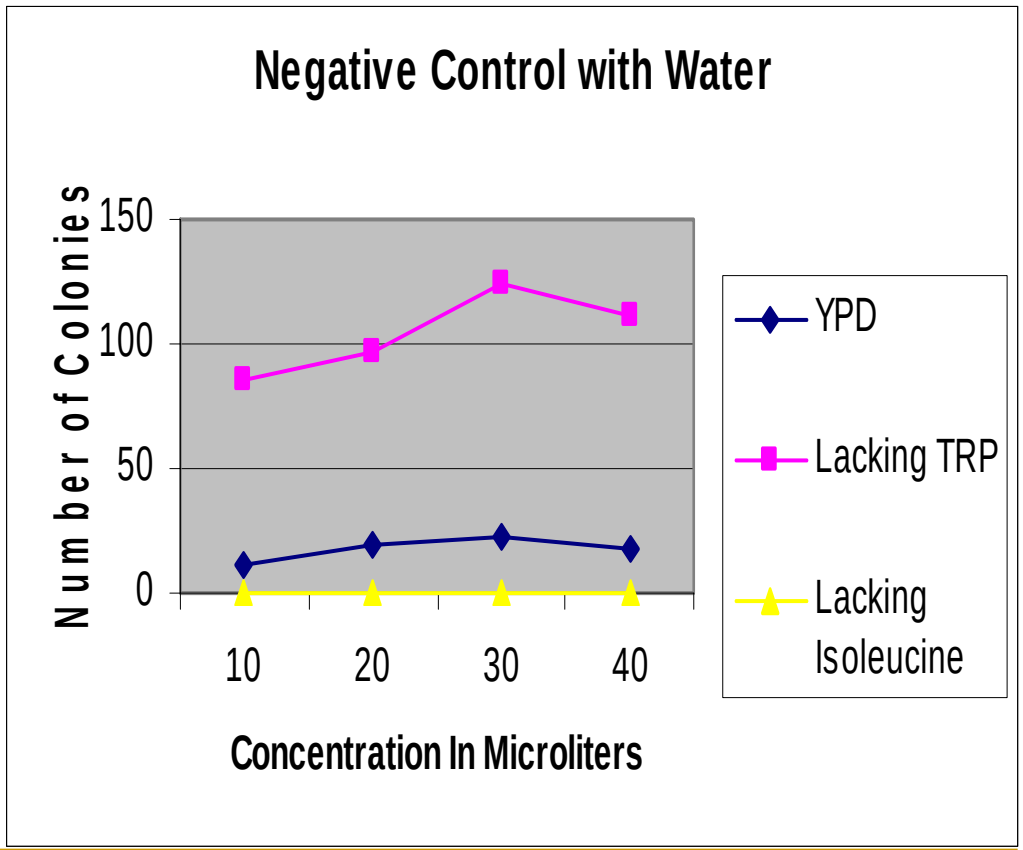
Concentration in µL	YPD (Pink)	Lacking TRP	Lacking ISO
0.5	65	120	0
1	53	150	0
1.5	37	210	1
2	25	360	1



Results

Negative Control with Water

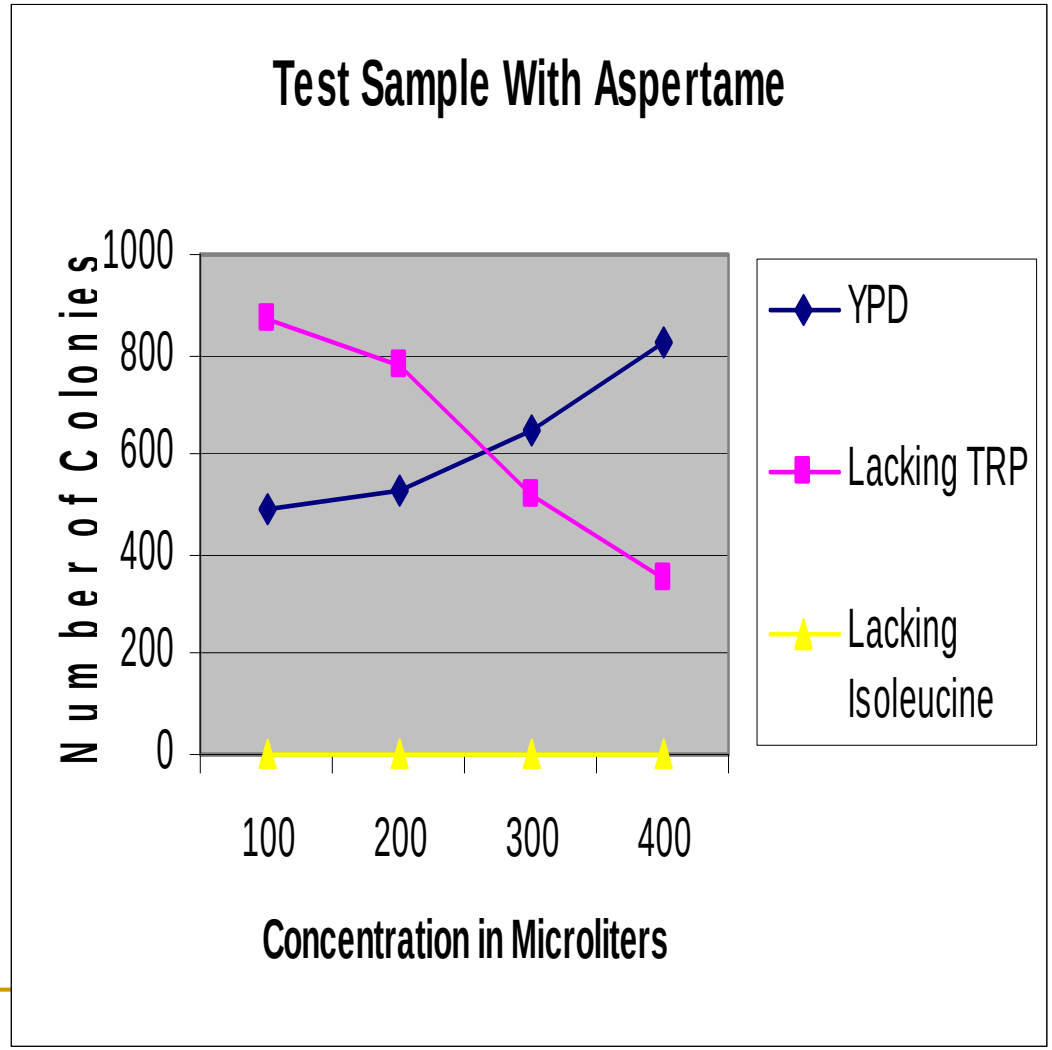
Concentration in µL	YPD (Pink)	Lacking TRP	Lacking ISO
10	12	85	0
20	20	97	0
30	23	125	0
40	18	112	0



Results

Test Sample of Equal Sweetener Containing Aspartame

Concentration in µL	YPD (Pink)	Lacking TRP	Lacking ISO
100	495	868	0
200	532	780	0
300	644	516	0
400	820	356	0



Discussion

- The results from this experiment supported the initial hypothesis that the compound aspartame is indeed mutagenic.
- Pink yeast colony growth on the YPD plate indicated mitotic crossing over.
- Growth on the -TRP plate indicated gene conversion
- Growth on the –ISO plate indicated a reverse point mutation
- The results for the test solution shows a steady increase for the YPD plate indicating it was mutagenic for mitotic crossing over
- The test solution also showed the –TRP colony count to be in a steady decline as the concentration of solution was raised. One explanation for this is that Higher levels of Aspartame on the –TRP plate bay be toxic to the yeast cells.

Discussion

- All of the plates lacking ISO had no yeast colony growth indicating that no reverse point mutations had occurred.
- The positive control showed significant growth on the –TPR plates as expected indicating
- The negative control showed very little growth which was expected for all plates
- In order to make this experiment more precise more trials with differing concentrations and saturation levels

Discussion

- ASP is considered a promising candidate to explain the increase in incident and degree of malignancy of brain tumors (Karikas et al. 1998)
- Many other studies still say there is not sufficient evidence to know is Aspartame is an actually source of caner or not.
- “More recent sustained increase in the incidence and shift toward greater malignancy that must be explained by some other factor and we conclude that the carcinogenic potential for aspartame should be reassessed (Olney et al. 1996)

Discussion

- Due to the high amounts of products containing ASP and the high amount of general consumption, the possibility of ASP being mutagenic could have disastrous results.
- Further, more extensive, and unbiased research on the effects of aspartame must be performed in order to determine if this household product is indeed a risk factor for illness such as cancer. Additional research would be beneficial in order to determine what amounts of ASP, if any, are safe to take on a daily basis without increasing the chance of cancer.

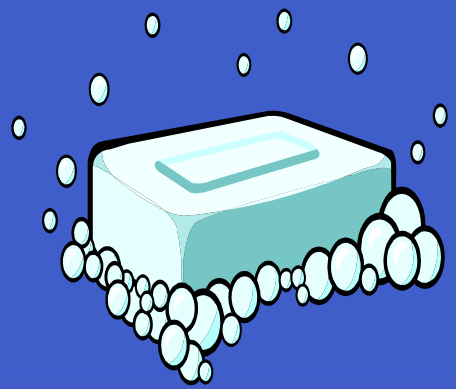
Literature Cited

- Karikas G. A., K. H. Schulpis, G. Reclos, and G. Kokotos. 1998. Measurement of Molecular Interaction of Aspartame and Its Metabolites with DNA. Clinical biochemistry **31**:405-407.
- Lim U., A. F. Subar, T. Mouw, P. Hartge, L. M. Morton, R. Stolzenberg-Solomon, D. Campbell, A. R. Hollenbeck, and A. Schatzkin. 2006. Consumption of Aspartame-Containing Beverages and Incidence of Hematopoietic and Brain Malignancies. Cancer Epidemiology, Biomarkers & Prevention **15**:1654-1659.
- Olney J. W., N. B. Farber, E. Spitznagel, and L. N. Robins. 1996. Increasing brain tumor rates: Is there a link to aspartame? Journal of Neuropathology & Experimental Neurology **55**:1115-1123.

http://www.mynewsmile.com/images/Crest_whitening_toothpaste.jpg

I Discovered that Crest Toothpaste is not Mutagenic

Introduction



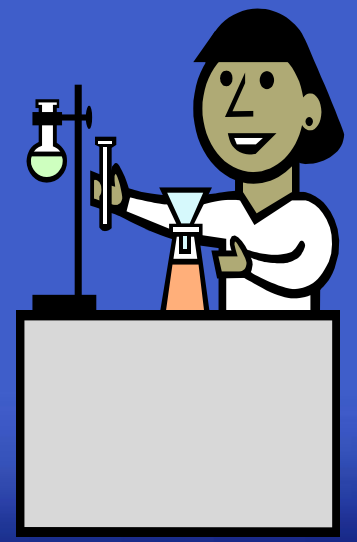
- Crest whitening cool mint gel toothpaste was used because one of its ingredients is Sodium Lauryl Sulfate.
- SLS can be found in most detergents, shampoos, and personal hygiene products. It is an ionic surfactant that is used for its thickening and lather ability
- Sodium Lauryl Sulfate (SLS) has been suspected to contain mutagenic properties in various sources.

Introduction

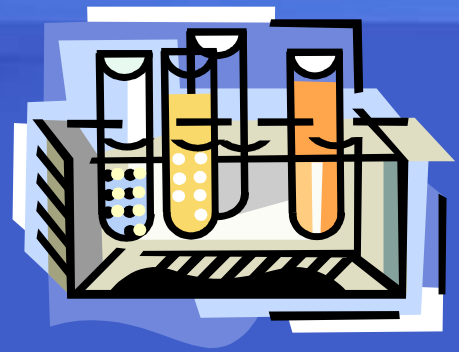
1. One article had a material safety data sheet which stated that tests on lab animals indicated mutagenic effects (Kramer, 2006).

2. A study by Fisher and colleagues indicated that SLS altered transforming growth factor β1 when applied to the skin (Fisher et al, 1992).

Hypothesis: The toothpaste will produce mutagenic effects on yeast cell growth.



Materials and Methods



Approximately 1 grams of toothpaste was mixed with 0.1 M Potassium buffer. Then the test tubes were prepared according to table 1.

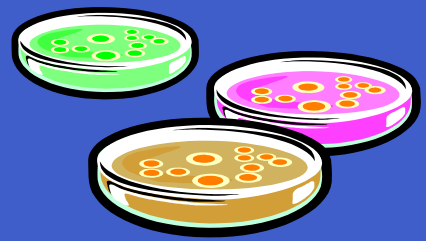
Test tubes were then placed in a 28°C water bath for 30 minutes.

Table 1. Test tube contents

Test tube	Yeast cell (ml)	Tooth-paste amount (ml)
1	0.3	0.1
2	0.3	0.2
3	0.3	0.4
4	0.3	0.8

Materials and Methods

- 100 µl of each test tube(1-4) was added to a YPD, Tryptophan lacking (TRP-), and Isoleucine lacking (ILE-) media plates.



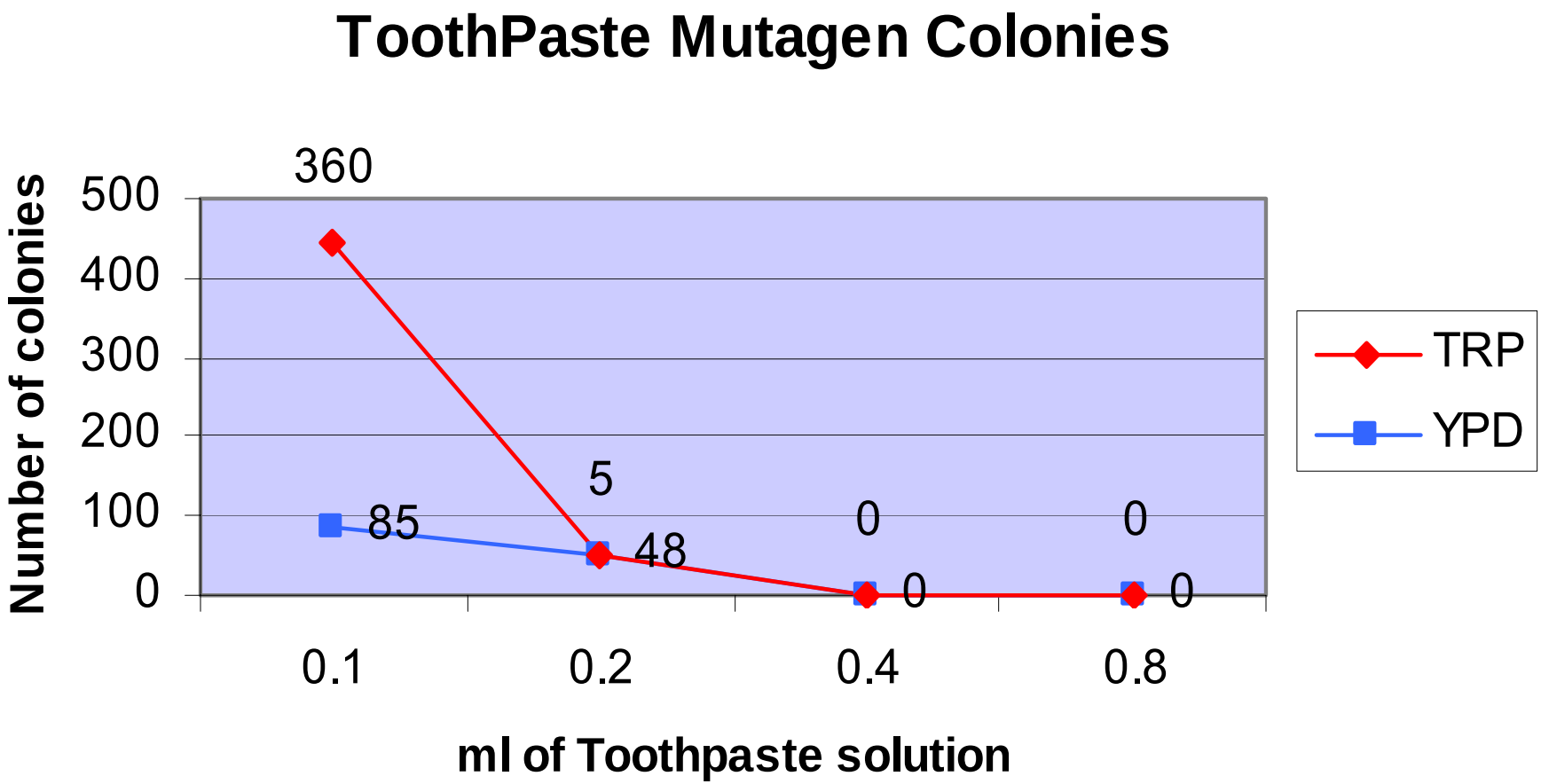
- 12 plates total
- Aseptic techniques with a hockey stick were used to smear each treatment onto the desired media.
- The plates were then allowed to incubate at 28°C for one week.

Results

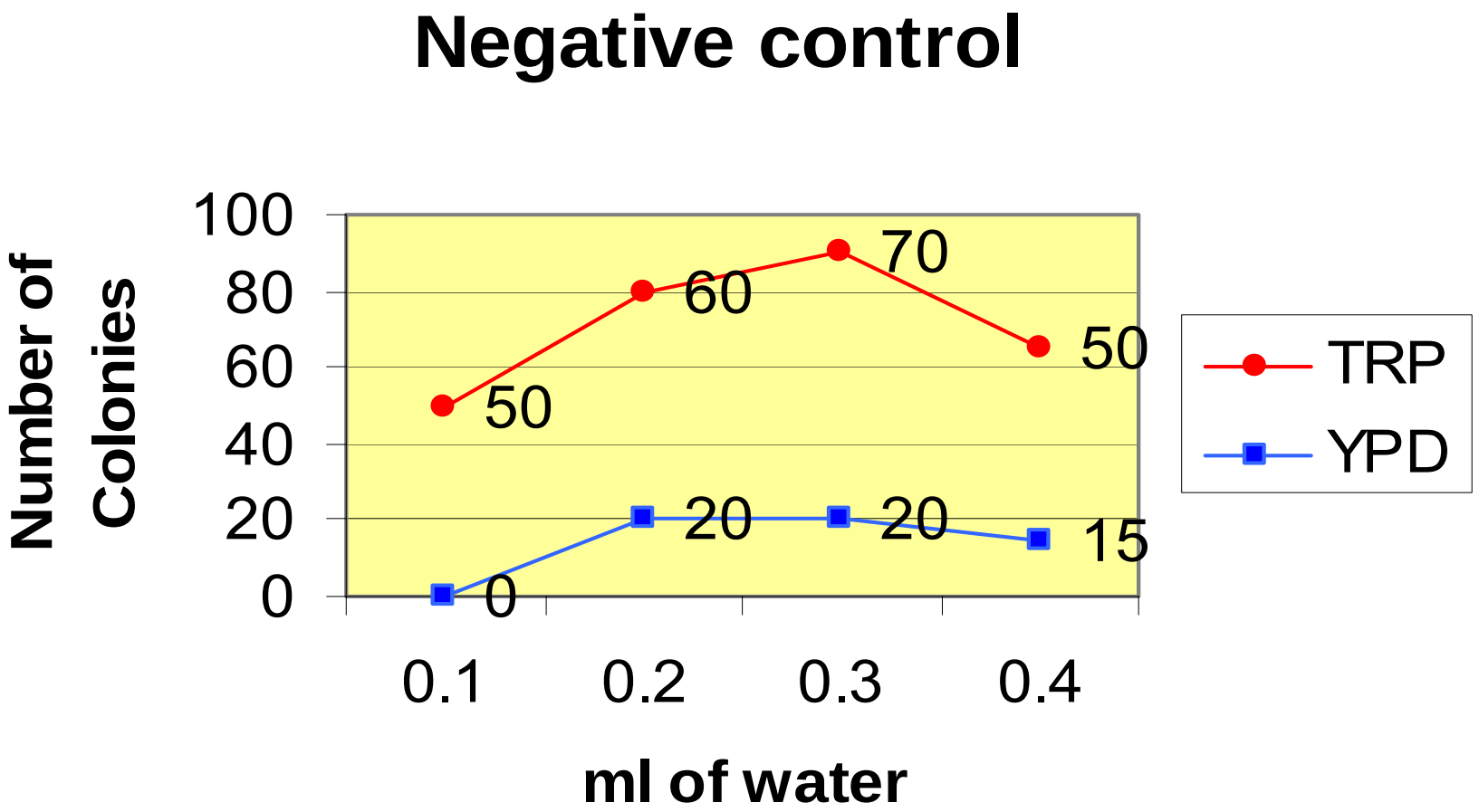
Table 2. Media plate Results with the number of Colonies formed

Toothpaste amount	ILE- (CFU)	YPD (CFU)	TRP- (CFU)
0.1 ml	0	85	360
0.2 ml	0	48	5
0.4 ml	0	0	0
0.8 ml	0	0	0

Results

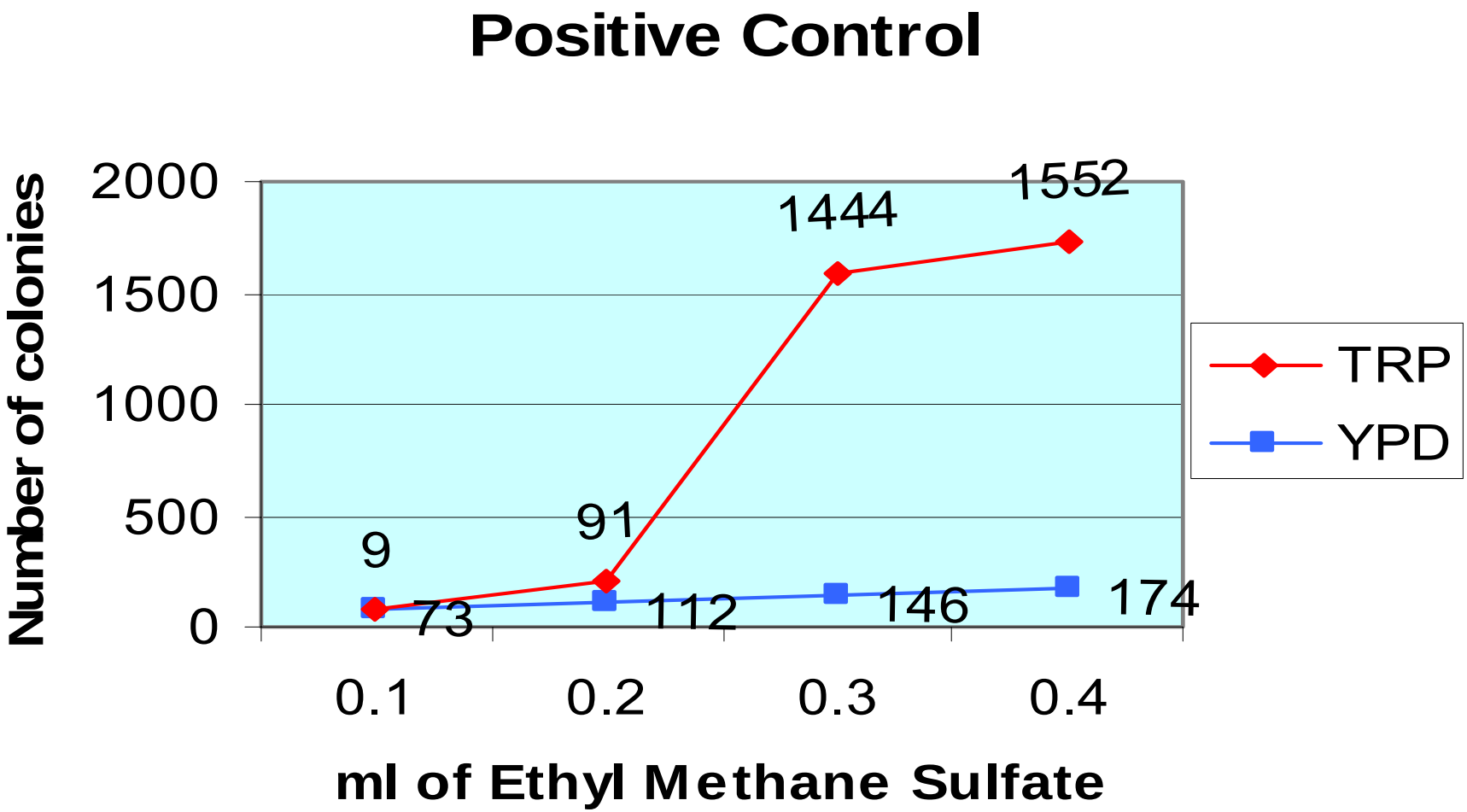

ToothPaste Mutagen Colonies
Number of colonies
500
400
300
200
100
0
360
5
85
48
0
0
0
0
0.1
0.2
0.4
0.8
ml of Toothpaste solution
TRP
YPD

Results- Control Graph 1



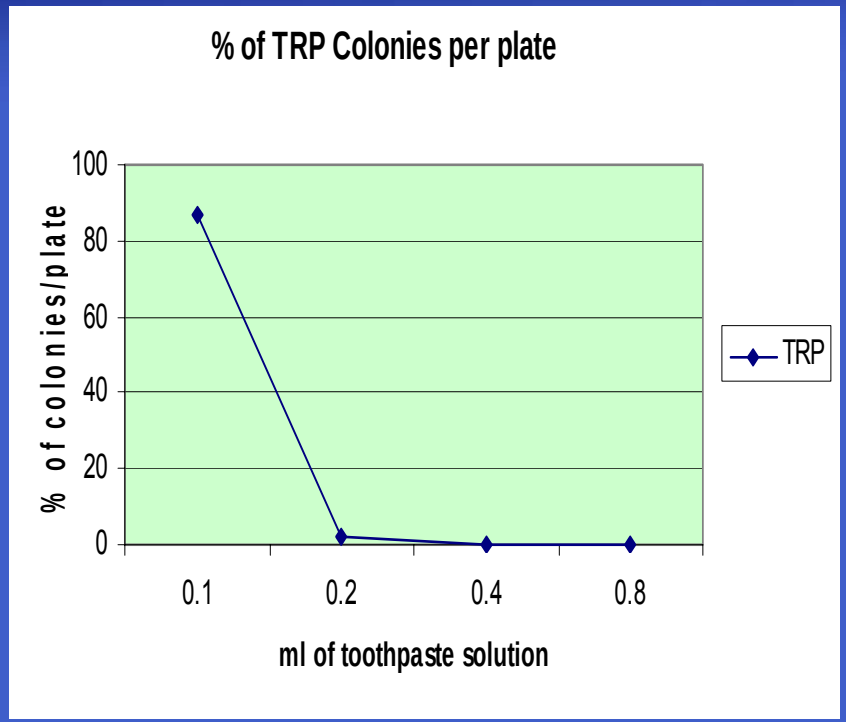
- There were no colonies formed on the ILE- plates

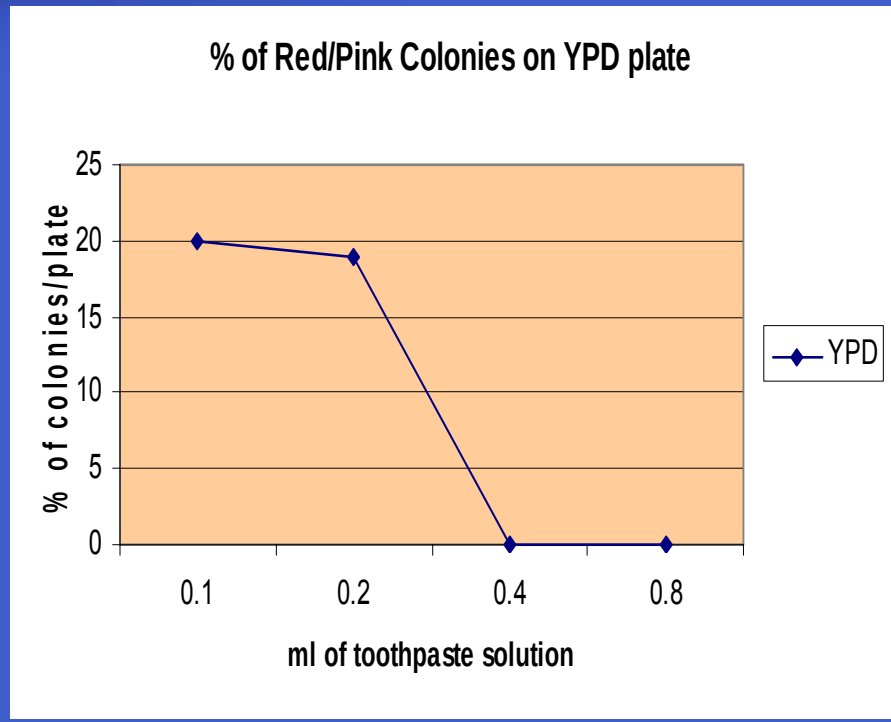
Results- Control Graph 2



-There were no colonies formed on the ILE-

Results- Survival %





The graph on the left is the % of Colonies per plate related to the number of colonies that are alive on the TRP- plate. The graph on the right is the % of red or pink colonies that formed per plate related to the number of colonies that are alive on the YPD plate.

Discussion

- *Comparing Negative Control:*

  The overall trend for my graph tends to slope down for both the TRP- and YPD plates. The negative control graph had sustainable growth that peaked around 0.3 ml and then declined.

- *Comparing Positive Control:*

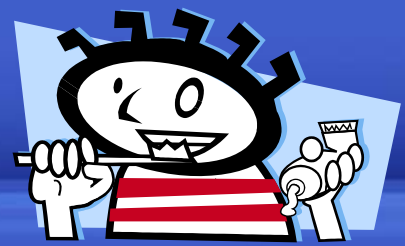
  The positive control graph increases exponentially at 0.3 ml and keeps increasing without any sign of immediate decline.

Discussion

Based on the comparison of the two control graphs, I believe that crest toothpaste does not produce mutagenic effects on yeast cells. The negative control graph was the closest representation of the colony growth displayed in my results. So, my hypothesis was rejected.

Discussion

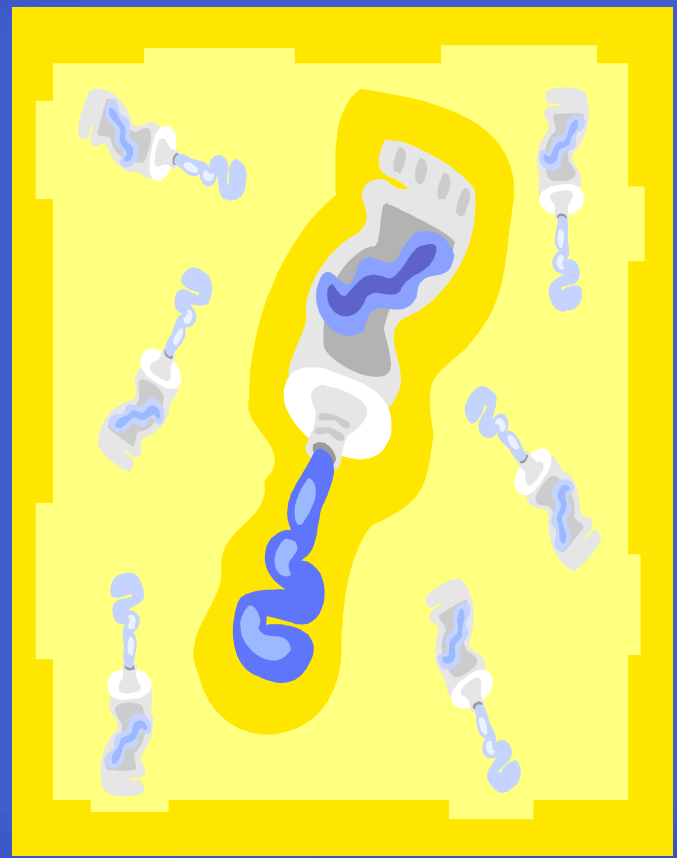
- The toothpaste concentration at 0.1 ml had a positive effect on the colonies formed because at that concentration, there was the highest growth in the yeast colonies.
- At 0.1 ml, there were 360 colonies formed on the TRP- plate and 85 colonies formed on the YPD plate.
- Both of these values(85, 360) are higher than the positive and negative controls' colony number's at 0.1 ml(0,50,9,73). Perhaps 0.1 ml of toothpaste provided some nutrients that the cells could use.
- I suspected toothpaste to be toxic at 0.4 ml because there was absolutely no growth on any of the plates. When I calculated the % of colonies formed per plate in relation to how many were still alive, the colony count went down drastically. Therefore, more colonies were killed as I increased my toothpaste concentration from 0.2 ml -0.4 ml.



Discussion

- Future research could repeat my experiment by decreasing the concentrations to a less toxic level. They can also investigate if there is a correlation with the toothpaste ingredients and possible nutrient sources for yeast cells.

- The application of my research is that there is no reason to fear SLS in toothpaste because the amount of ingredient in crest toothpaste does not produce any damaging effects. Toothpaste does have many ingredients but the interaction of toothpaste SLS on cells will not harm anyone.



References

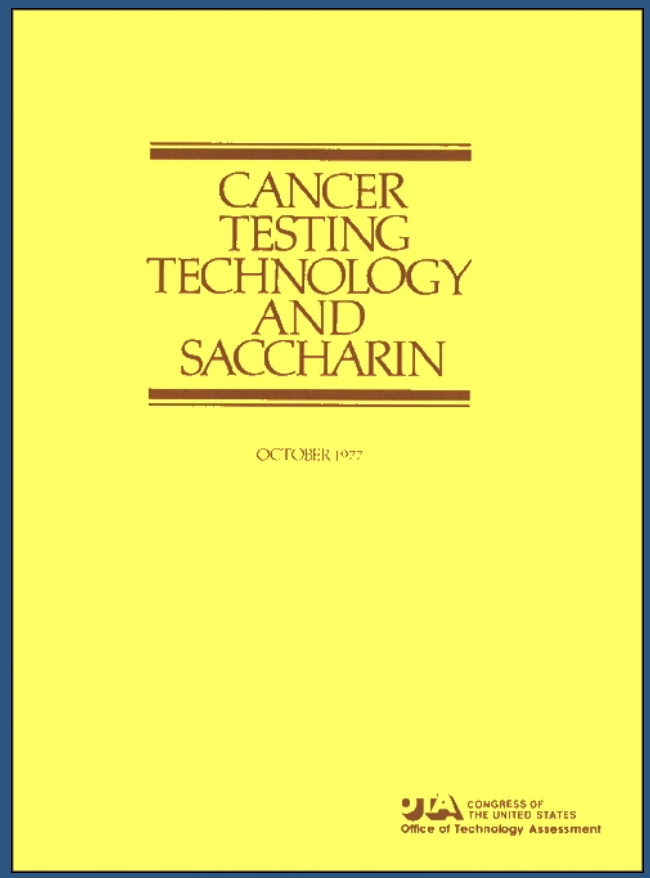
Fisher, G.J. (1992) Differential Modulation of Transforming Growth Factor- β1 Expression and Mucin Deposition by Retinoic Acid and Sodium Lauryl Sulfate in Human Skin. Journal of Investigative Dermatology. **98**:102-108

Kramer, S.R. (2006)http://www.healthy-communications.com/msdssodiumlaurylsulfate.html

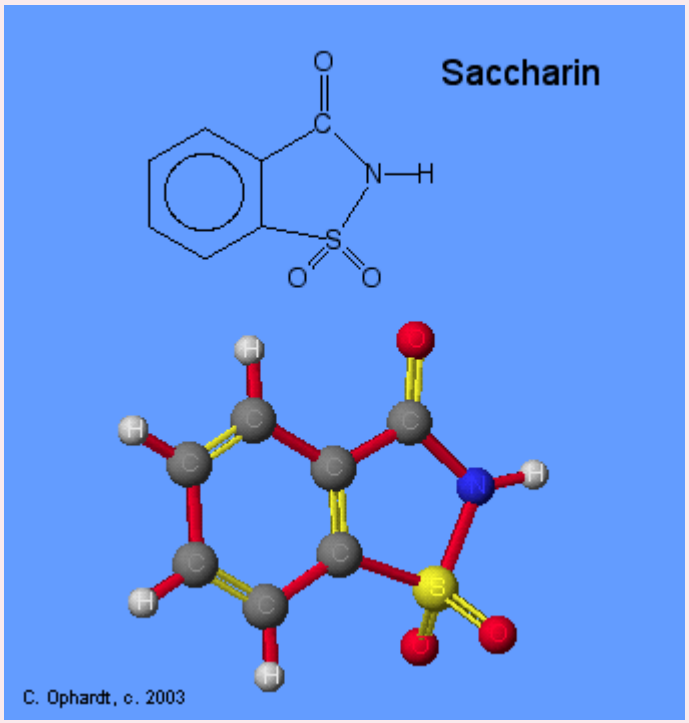
Mutagenic Activity of Saccharin on Yeast (*Saccharomyces cerevisiae*)

Introduction:

- For this lab, I chose to test Sweet n Low as a possible mutagen.
- According to FDA Consumer, in 1977, the FDA proposed a ban on saccharin because of potential health risks (cancer) that developed in laboratory test rats after receiving increased dosages of saccharin.
- At that time, Congress passed the “Saccharin Study and Labeling Act”.
- However, since then, saccharin has been taken off the list of” potential cancer-causing agents”, as well as, the labeling of saccharin as a potential carcinogen.
- According to an article posted by the Center of Science in the Public Interest (CSPI), a study was published by the National Cancer Institute, that stated the incidence of bladder cancer rose more than ten percent between 1973-1994.



Introduction: (cont.)



- The National Cancer Society found that there was an association between artificial sweetener consumption and bladder cancer, specifically those that had greater consumption.
- In addition to the NCS study, the National Toxicology Program tested the possible mutagenic affects of saccharin on various animals, they were able to support their hypothesis that saccharin is mutagenic.
- The results showed that saccharin was found to be “weakly mutagenic” (at “low doses”) to Drosophila, hamsters, mice, and even Salmonella.

Introduction: (cont.)

With information obtained through various websites, regarding the possible effects of saccharin, I was unsure and curious as to whether it was harmful or not.

Most of the studies performed stated that saccharin was a potential carcinogen, but others stated that low doses were not harmful.

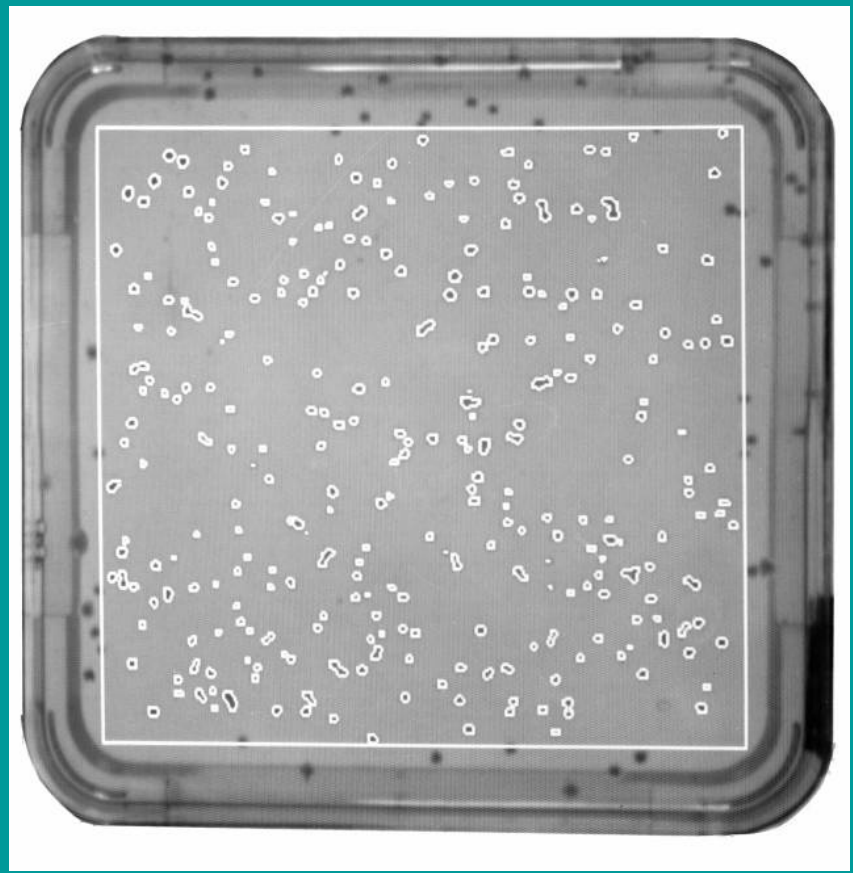
The various reports led me to my hypothesis that saccharin is a mutagenic compound.

Materials & Methods

- Twelve pre-made yeast cell petri dishes were obtained and labeled as follows:
  1-4 (YDP), 1-4 (-Trp), and 1-4 (-Iso).
- 3.0 grams of Sweet n Low was weighed and 2.0 mls of water was added and mixed to make a super-saturated solution.
- The petri dishes labeled #1 were obtained and 100 microliters of the super-saturated solution was added to each, and mixed with a sterile glass rod, which was dipped in ethanol and warmed.
- The petri dishes labeled #2 were prepared in the same manner, however, 200 microliters of super-saturated solution was added.
- Petri dishes labeled #3 were obtained and prepared in the same manner with the exception of using 300 microliters of super-saturated solution.
- Finally, petri dishes labeled #4 were prepared in the same manner, using 400 microliters of super-saturated solution.

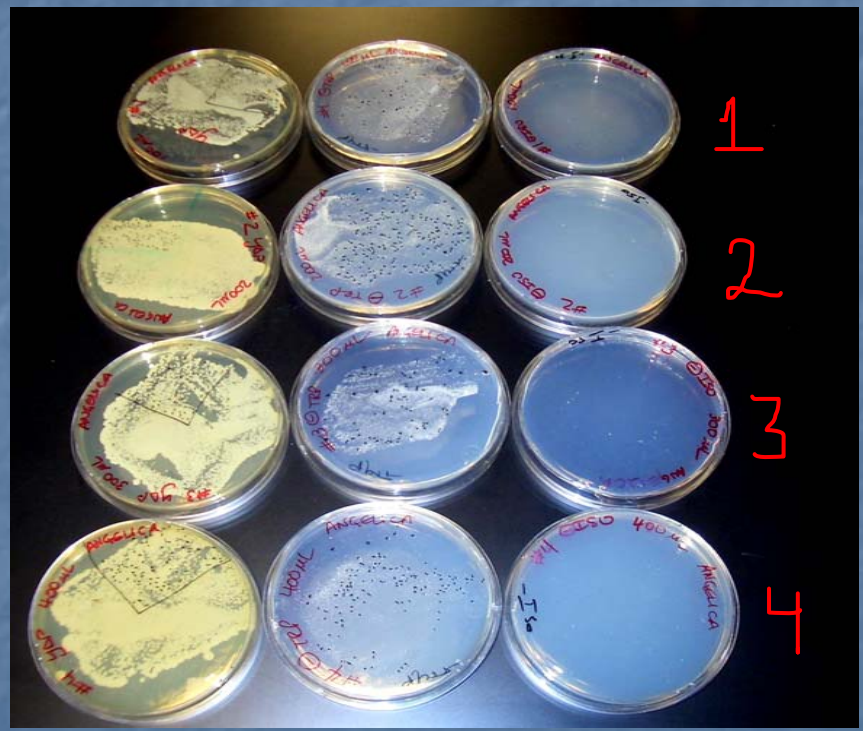
Materials & Methods (cont.)

- The controls for the experiment were as follows: the negative control (water) was placed in increasing amounts into each of the petri dishes labeled 1-4 of the YDP, -Trp, and –Iso in increasing amounts (100 microliters-400 microliters, respectively). The positive control (EMS) was placed in increasing concentrations of 0.5%, 1%, 1.5%, and 2% into each of the petri dishes as noted above. The controls were prepared by Dr. Marshall.
- All petri dishes were incubated for one week at approximately 28 degrees Celsius.
- After one week of incubation, phenotypes of colonies were observed on each of the plates and compared to the controls.



Results:

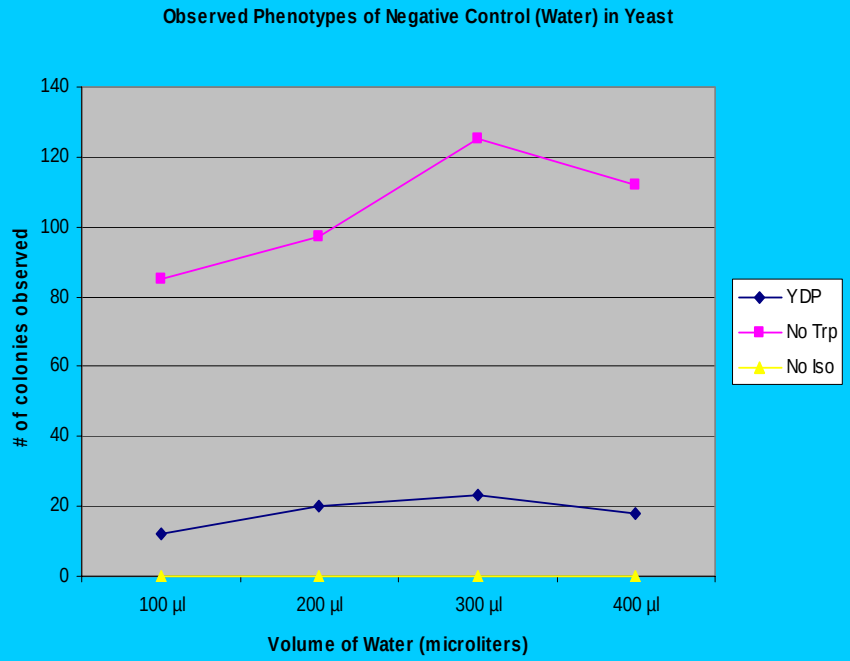
- The petri dishes on the left are the YDP plates and are evident of growth. These appeared to have the most growth on them. Although they are not easily seen, there was evidence of reddish/pink colonies on each of the plates.
- The petri dishes in the middle are the –Trp group and also have evidence of growth. These were evident of white colonies only.
- The petri dishes on the right are the –Iso plates and completely lack growth. There appear to be a couple of small colonies on the #3 plate, however, they are only air trapped beneath the medium.

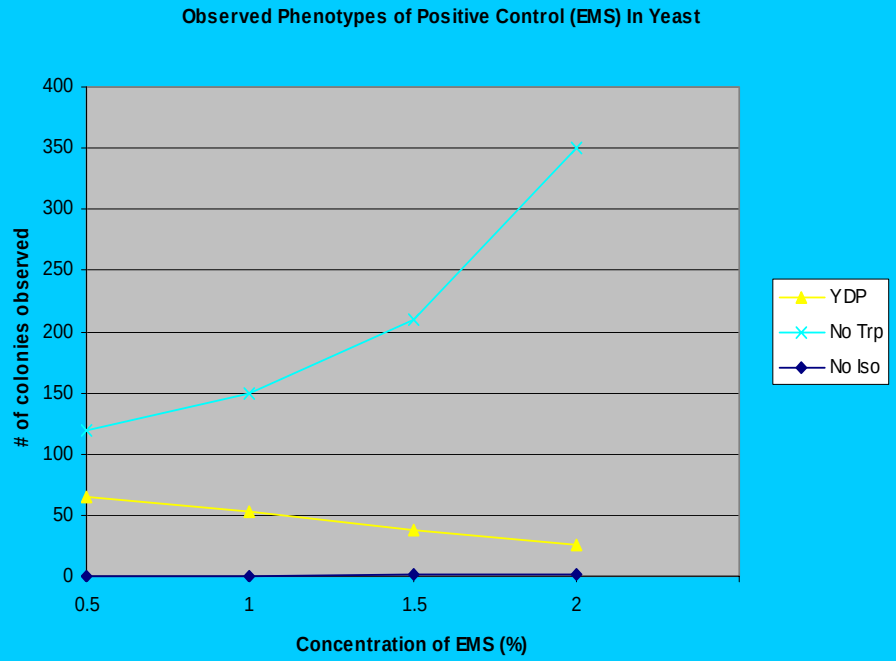


Results (cont.)

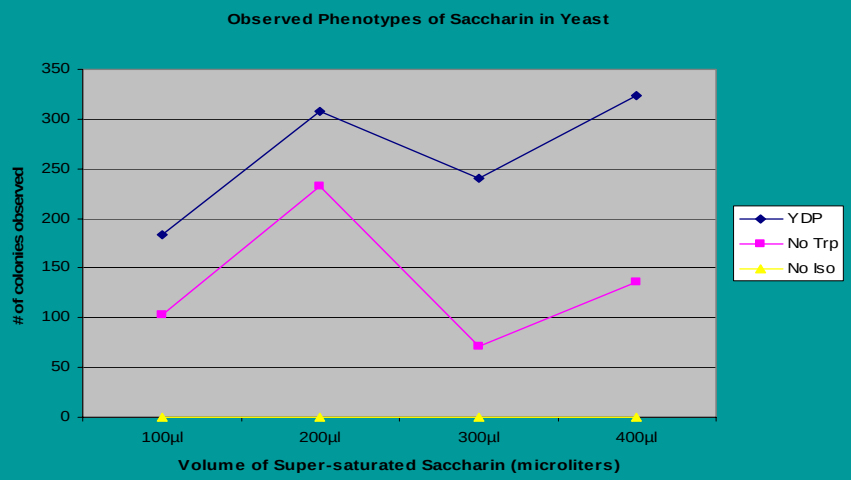
The graph illustrated on the bottom left is the graph of the negative control (water) and the phenotypes observed on each petri dish. The most colonies observed were in the -Trp petri dishes. The least colonies observed were those in the –Iso petri dishes.

The graph illustrated on the bottom right is the graph of the observed phenotypes of the positive control (EMS). The –Trp dishes had the most colonies and the –Iso dishes had the least amount of colonies.





Results (cont.)



This graph represents the data obtained from my testing of saccharin on the various media.

The phenotypes observed were as follows: the YDP plates had the most colonies, the –Trp had the second most. Finally, the –Iso had the least or no colonies.

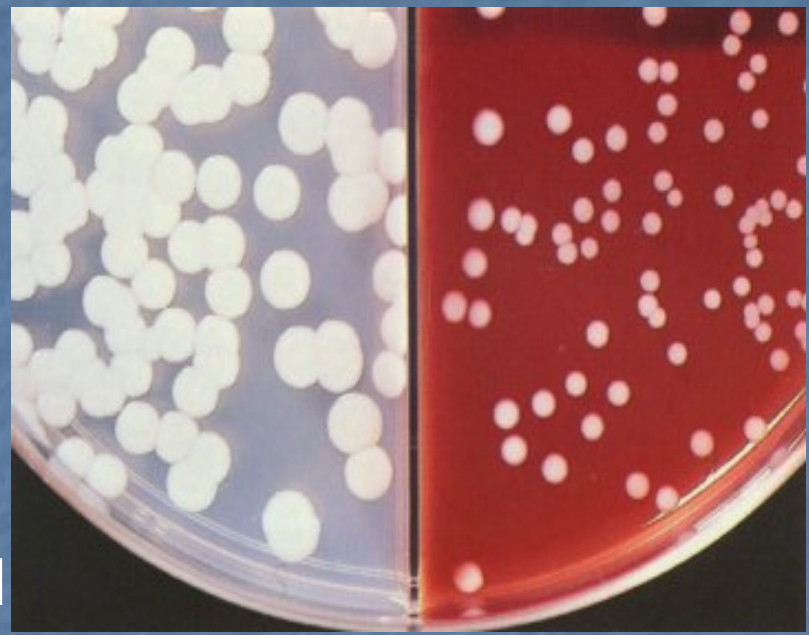
The phenotypes observed in the YDP dishes were both white and reddish/pink colonies.

The phenotypes in the –Trp plates were the white colonies only.

There were no colonies present on any of the –Iso dishes.

Discussion:

The presence of colonies on petri dishes lacking specific enzymes (in this case, Trp and Iso) suggests that a suspected mutagen causes mutation.



The colonies that formed on the -Trp dishes helped to conclude that the DNA had been repaired by means of mitotic gene conversion.

The lack of colonies on the –Iso dishes helped me to conclude that no mutation had occurred on these dishes.

Discussion (cont.)

- There was evidence of large “lawn” growth on the YPD dishes. Compared to the negative control, the observation of red and pink colonies (in addition to the white) helped to support the hypothesis that Sweet n Low was mutagenic.
- The low number of colonies on the negative control (12-23) was significantly less than those observed on my petri dishes, with the least number of colonies observed as 184. This lead me to believe that recombinant genetic repair had occured.
- The hypothesis that saccharin is a mutagenic compound was supported with the presence of colonies on the YPD dishes as compared to the negative controls and the presence of colonies on the –Trp petri dishes compared to the negative controls.

Future Research:

- The danger of mutagens is important to the well-being of people worldwide. Research is conducted on animals due to the ethical issues on the testing of humans and the implications that they cause.
- If tested in future experiments, I would like to take my experimentation to the next level and utilize animal models to test whether or not the compound is destructive to DNA.

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Your Morning Coffee Is Mutagenic!

NOT MY COFFEE!

- The chemical I brought in to test was caffeine, also the main fun ingredient in your favorite drinkable meal. A strong 12 ounce cup of coffee contains about 200mg of caffeine.
- I brought caffeine in the form of a pill, and each pill contains 200mg of caffeine.
- Commonly sold as No-Doz, all generics contain the same amount of caffeine per pill.

Now what would make you say a terrible thing like that?

- Friedrich Pons and Pia Muller of the Johann Wolfgang Goethe University's Institute for Microbiology found that caffeine induced frameshift mutations in E. Coli Bacteria
- Caffeine meets the University of Maryland definition of a **Mutagen** for the purpose of the Chemical Hygiene Plan. It was listed as a mutagen in the "Dangerous Properties of Industrial Materials", 7th Ed., by N. Irving Sax and Richard J. Lewis.

Now what would make you say a terrible thing like that?

- Anita Kluczna and Hieronim Bartel of *Department of Histology and Tissue Ultrastructure, Medical University* in Poland state that caffeine “likely mutagenic” and teratogenic, because it can act like the adenine base.
- A. Nehlig and G. Debry published a PubMed article that states that caffeine is mutagenic to bacterial and fungal cells, as well as mammalian cells at high concentrations.

Now what would make you say a terrible thing like that?

- Based on these previously published articles, my hypothesis was that the caffeine would produce mutagenic effects in the Saccharomyces Cerevisiae.
- That means I expect to see white growth on TRP- and ILE- plates, and red/pink colonies among white growth on the YPD plates.

So, what now?

- Now we design an experiment that will determine if caffeine is indeed mutagenic to S. Cerevisiae (yeast) cells.
- Since I used caffeine pills (and not a liquid), I had to create a solution to most accurately measure different concentrations since each culture would have different concentrations of the suspected mutagen. I placed finely crushed caffeine powder into a phosphate buffer and made a super-saturated solution. At that point, I centrifuged the tube to get all the leftover precipitate to the bottom to keep from pipetting it into my yeast (again, gives a more uniform measure of concentration).

So, what now? Let's mutate some yeast!

- After making the solution, we first set up our 'yeast tubes'. You will have 4 total tubes, each containing 300µL of yeast cells.
- Tube #1: Add 100µL of caffeine solution.
- Tube #2: Add 200µL of caffeine solution.
- Tube #3: Add 300µL of caffeine solution.
- Tube #4: Add 400µL of caffeine solution.

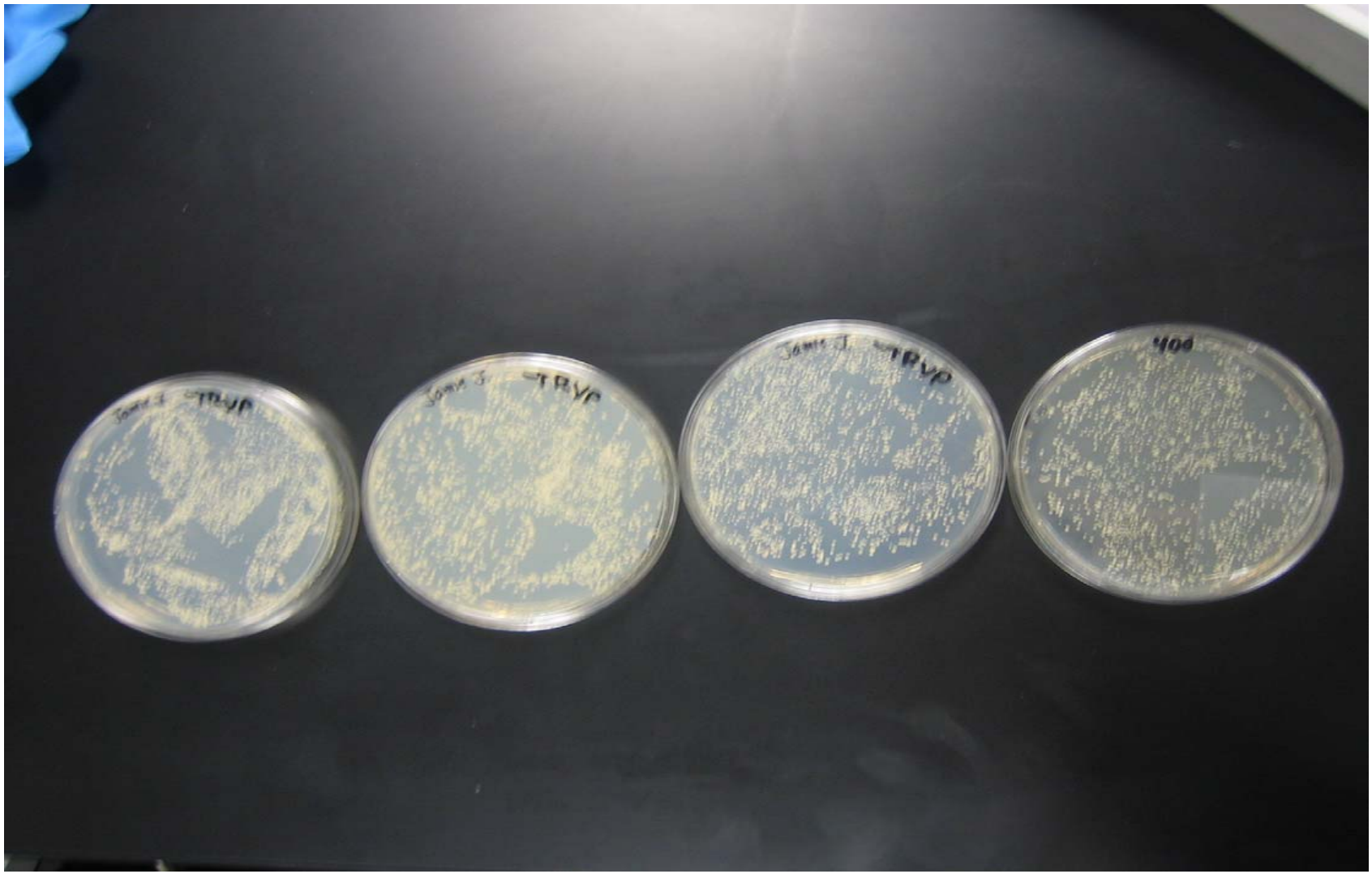
So, what now? Let's mutate some yeast!

- The setup includes 12 total plates with 3 different medias.
- Four of the plates contain a complete media (YPD plates).
- Four of the plates contain a media lacking Tryptophan (TRP-).
- Four of the plates contain a media lacking Isoleucine (ILE-).
- Four plates of YPD/TRP-/ILE- and four different concentrations of suspected mutagen solution…hmm…100µL of cells/solution on each!
- 1 YPD plate = 100µL of 100µL concentration, 1YPD plate = 100µL of 200µL concentration, 1 YPD plate = 100 µL of 300µL concentration, 1 YPD plate = 100µL of 400µL concentration
- (and the same with the TRP an ILE plates)

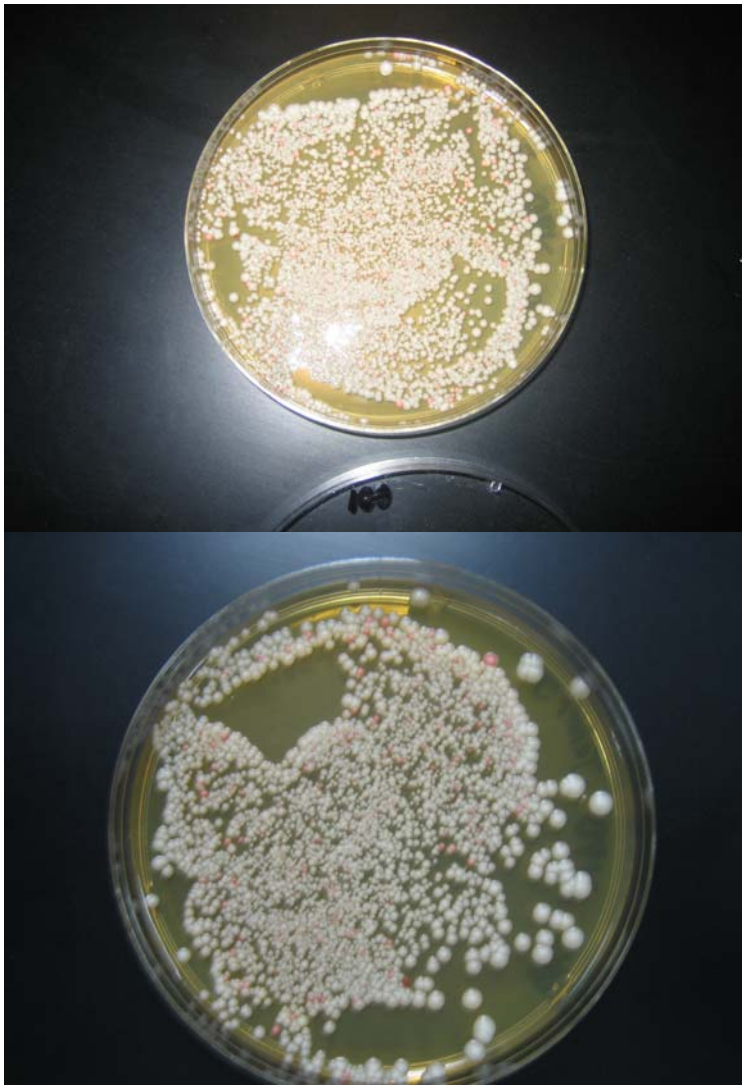
So, what now? Let's mutate some yeast!

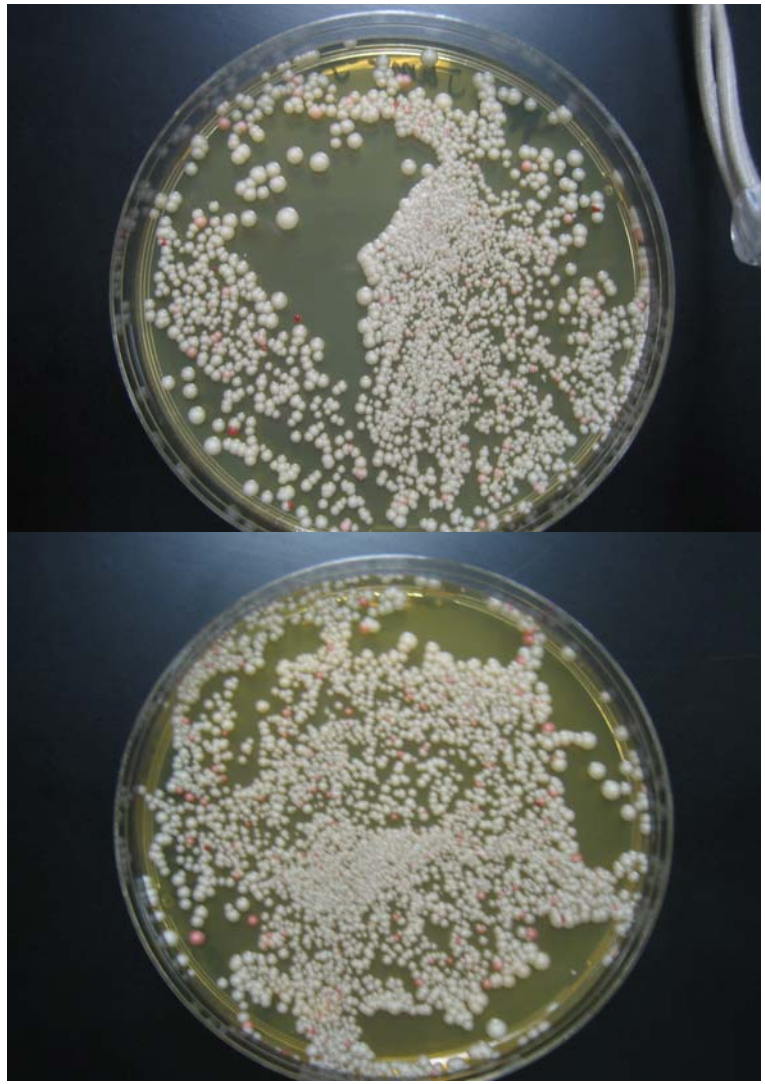
- An important part of this lab is also the positive and negative controls.
- We use water as a negative control. There will always be *some spontaneous* mutations in all cultures. So if the negative control plate has 5 mutated colonies and I did too, caffeine probably isn't mutagenic at that concentration.
- We use a known mutagen as a positive control to make sure that our assay is working correctly. In this experiment we will use Ethyl Methane Sulfate, which induces crossing over and gene conversion mutagenesis .

The Proof Is In the Plates: TRP-

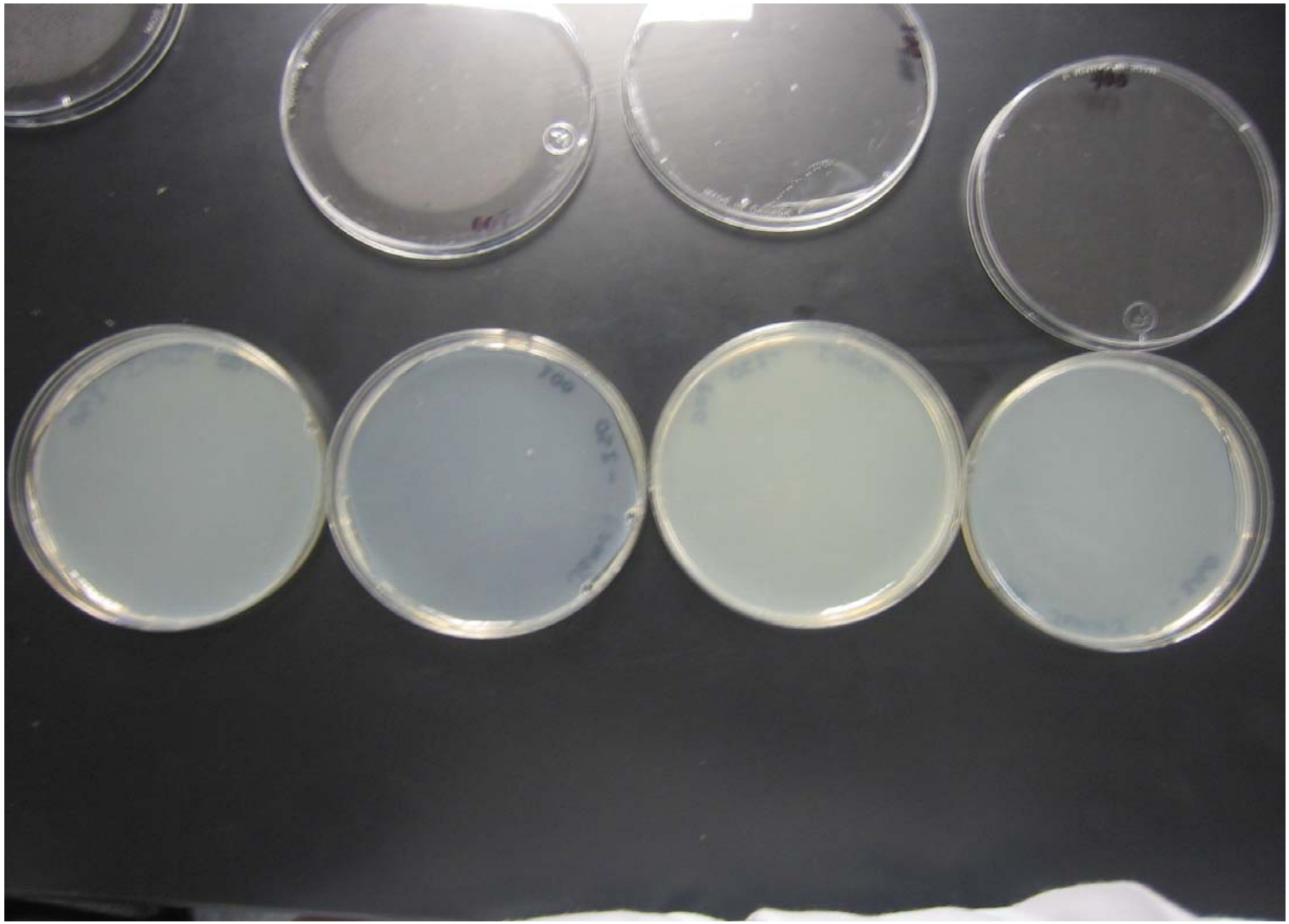


The Proof Is In the Plates: YPD

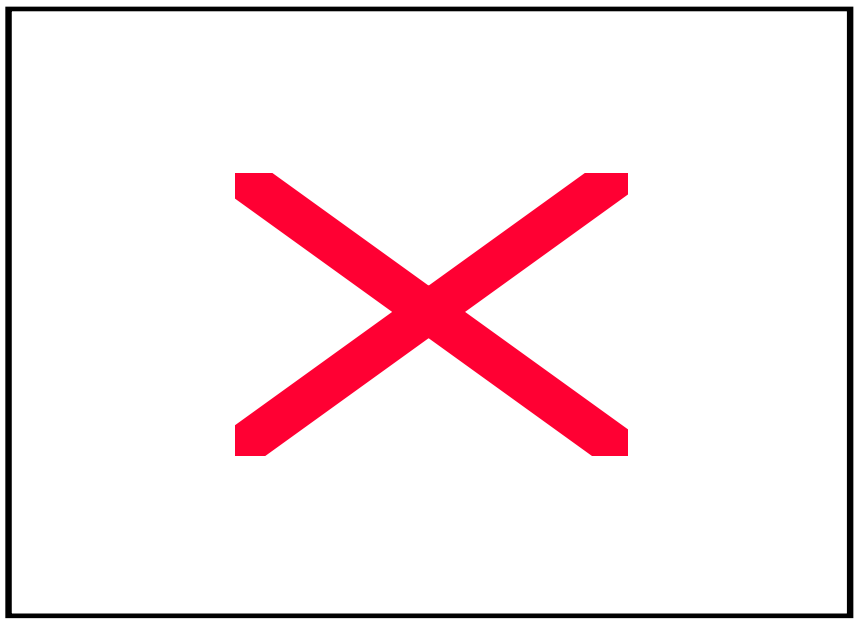


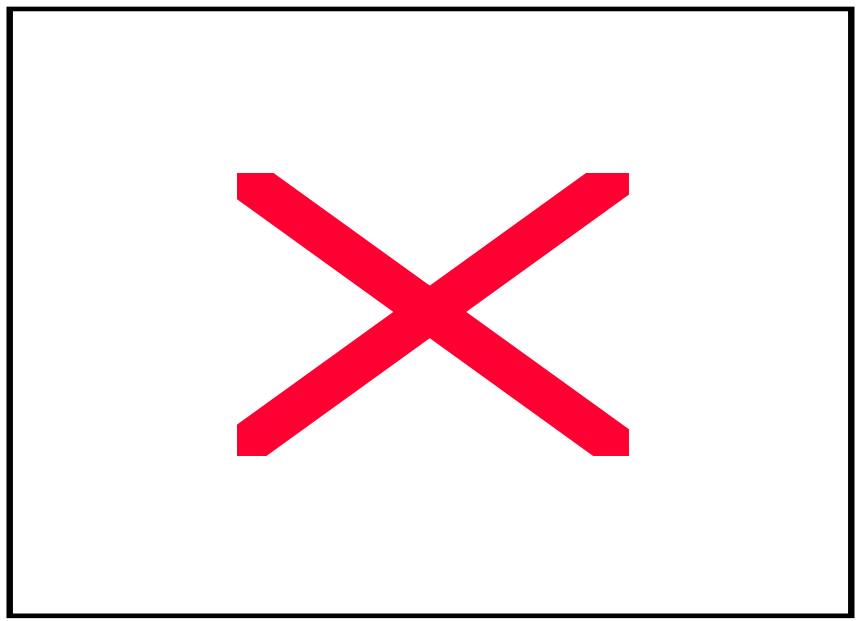


The Proof Is In the Plates: ILE-

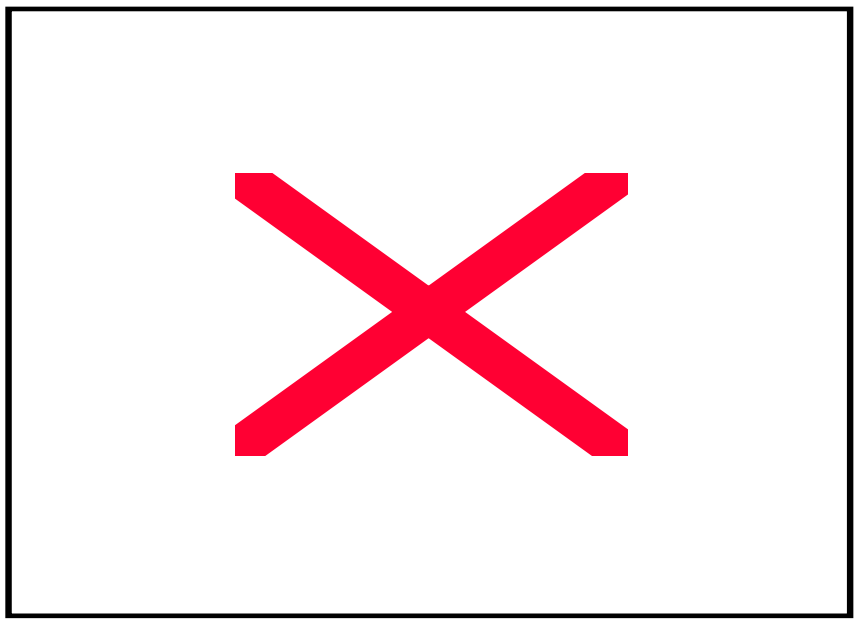


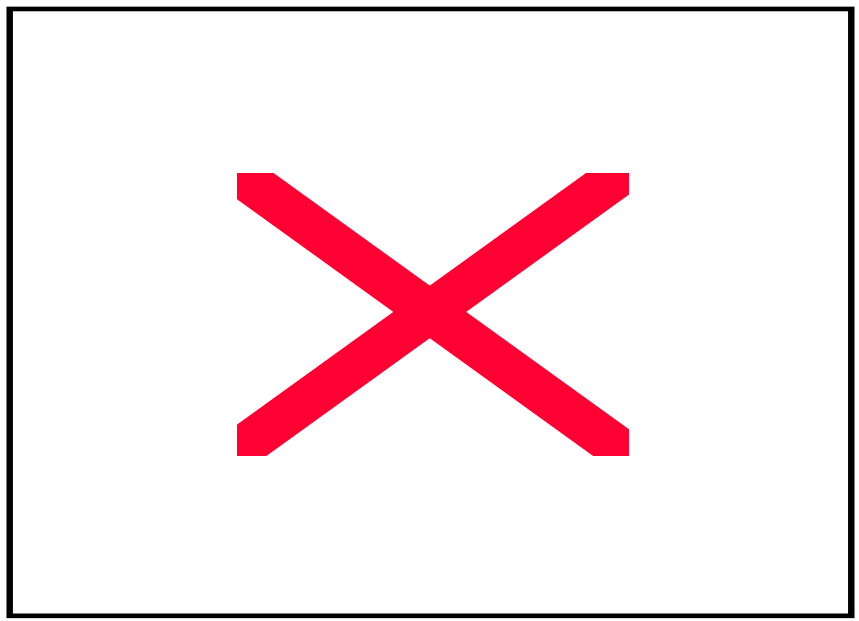
Results Graphs (Same Graph, two scales)





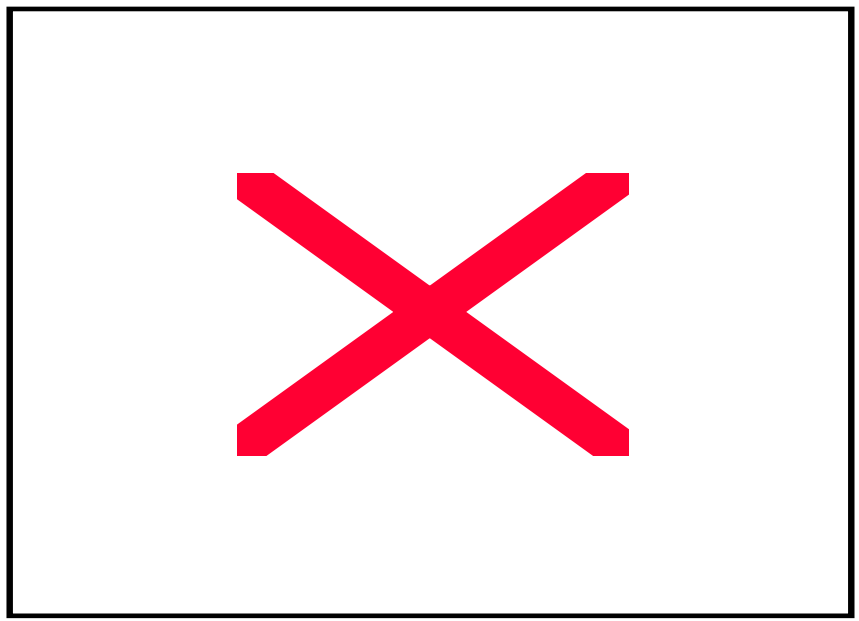
Positive Control Results Graphs





Negative Control Graph

- This graph may look similar to the others, but pay close attention to the Y-axis scale. The number of colonies doesn't exceed 70 on any of the plates. Also, you can notice that the number of colonies stays relatively static when compared to the test compound and the positive control graphs.



What does this have to do with my morning coffee?

- Red and Pink colonies on the YPD plates are indicative of mutation via mitotic crossing over.
- Growth on the TRP- plates is indicative of mitotic gene conversion.
- Growth on the ILE- plates is indicative of reverse (point) mutation.
- My results showed red/pink colonies on YPD plates as well as growth on the TRP- plates (that increased with caffeine solution concentration), but no growth on any ILE- plates.
- This means my hypothesis is likely correct – caffeine is mutagenic to this strain of yeast cells!

What does this have to do with my morning coffee?

- The YPD plates had the number of red/pink colonies increase from 100µL to 300µL, then a *decrease* from 300µL to 400µL. A likelihood in this case is that at certain levels of concentration, caffeine is actually lethal to the yeast. A similar effect was observed with the TRP- plates.
- The ILE- plates had no growth at any concentration. What does this mean? This means that like our positive control (ethyl methane sulfate) , caffeine probably doesn't induce reverse (point) mutations. The ILE- plates also had no growth on the positive control plates for this same reason.
- So, now should you stop drinking coffee or continue at your peril?

There’s good news!

- The good news is here: it’s kind of inconclusive!
- To make this experiment a little more specific to the purpose of discovering if it’s harmful to us, we should probably include some liver enzymes, or similar enzymes that the human body uses to break down substances. The fact is, when we consume something, it doesn’t go right to work destroying our cells.
- Also, in a future experiment I might suggest using a *little* less of the caffeine solution. For instance, I saw exponential growth at 100µL on the TRP- plates – it makes for difficult counting, and the question of lethality hangs over you once you get up to 400µL concentration. Was it really lethal? Or did you accidentally kill some cells while plating?

There’s good news!

- Future testing also might dictate trying to plate on media lacking other different amino acids.
- Also, it’s important to note that this particular lab leaves a lot open to human error: keeping sterile plating procedure, counting colonies with the naked eye, eyeballing solutions, etc.
- Next time, to concentrate further on the effect of caffeine, it might be a good idea to see if you could further isolate caffeine since caffeine pills do have other small ingredients.
- Moral of the story – don’t cry over your Starbucks tomorrow.

Citations

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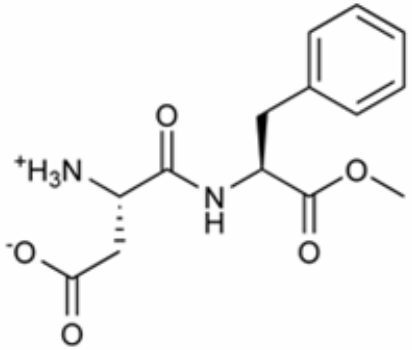
I Determined that Diet Pepsi® is a Mutagen

Introduction



- Aspartame is an artificial and non-carbohydrate sweetener introduced in 1981 in USA
- First discovered in 1965 by Dr. James L. Schlatter while synthesizing an anti-ulcer drug for G.D. Searle & Company
- Aspartame contains 4 kilocalories per gram and it is about 200 times sweeter than sugar
- Only small amount of aspartame is required to produce a sweet taste and hence the caloric input is insignificant
- Aspartame is a popular sweetener for those trying to avoid calories from sugar and for diabetics

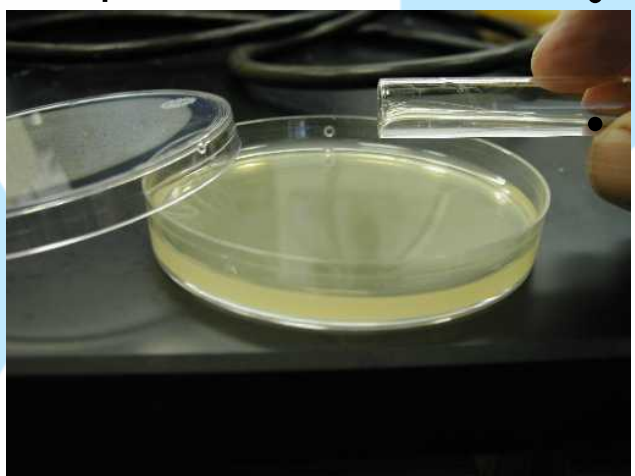
- Aspartame is sold under trademark names such as: Equal®, NutraSweet®, and Canderel®
- Commonly used in diet soft drinks, sugar-free gum, and chewable vitamins
- According to the Cancer Research Center of the European Foundation of Oncology aspartame “induces an increase in lymphomas and leukemia in female rats”
- Neurosurgeon Dr. Russell Blaylock M.D. commented that “Both of these malignancies have increased significantly in this country since the widespread use of aspartame”
- Diet Pepsi® post mix syrup contains concentrated amount of aspartame and will be a carcinogen for *S. cerevisiae* cells

The NutraSweet Company

CANDEREL

Materials

- 500 µl of Diet Pepsi® post mix syrup
- Yeast cell culture in 0.1 M potassium phosphate buffer with pH 7 in 1/100 dilution
- 4 YPD media plates
- 4 media plates lacking tryptophan
- 4 media plates lacking isoleucine
- Ethanol and glass spreader
- Bunsen burner
- Micropipettes
- Pipette tips



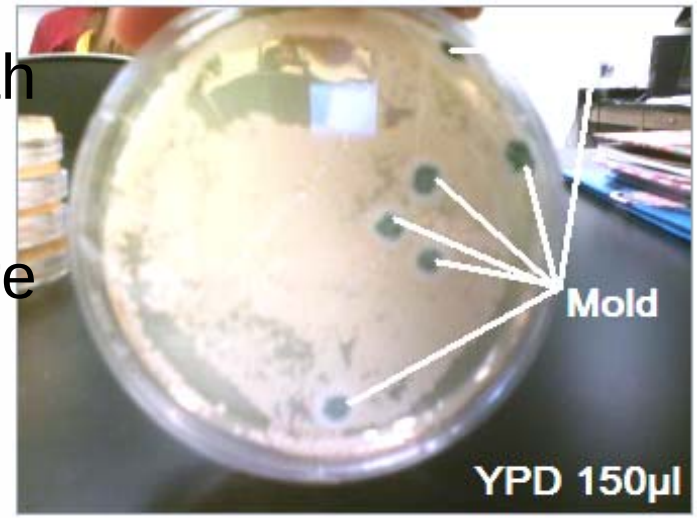
Methods

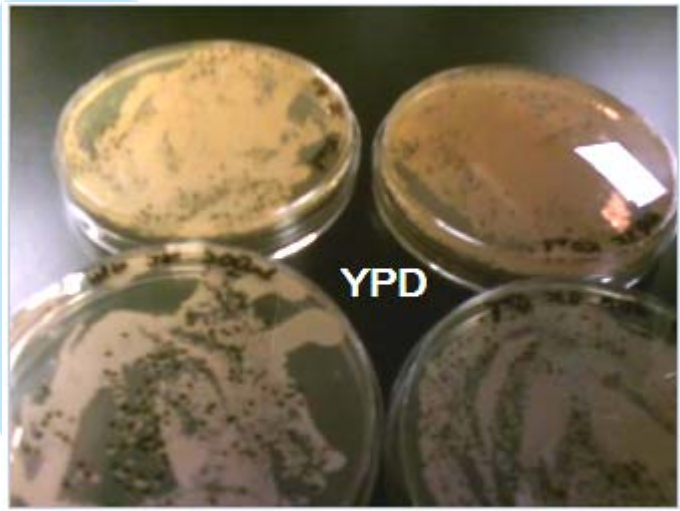
- In four test tubes containing 300 µl of cells, 50 µl, 100 µl, 150 µl, and 200 µl of Diet Pepsi® post mix syrup was added
- Tubes were under treatment for 30 minutes
- 100 µl of each different treatment was spread on YPD, – TRP, and – ISO media plates
- Plates were incubated at 28^{o} C for one week
- Phenotypes of yeast cells were observed and data was collected

- Negative control with 100 µl, 200 µl, 300 µl, and 400µl of water and Positive control with 0.5%, 1.0%, 1.5%, and 2.0% of EMS was used following the same method.
- Negative control was used to confirm that the yeast cells were not contaminated and to observe how the yeast cells grew in absence of a mutagen
- Positive control was used to observe how the yeast cells grew in presence of a mutagen and to refer the phenotypes of yeast cells from the test compound
- Phenotypes and the number of growth of the yeast cells from the controls were compared to the yeast cells from the test treatments

Results

- In the YPD plates, there were lawns of yeast cell growth and pink cells
- In the 150 µl plate, there were six mold, about one cm in diameter were growing on the plate
- In the plates lacking tryptophan, there were cell growth
- In the plates lacking isoluecine, there were no cell growth.





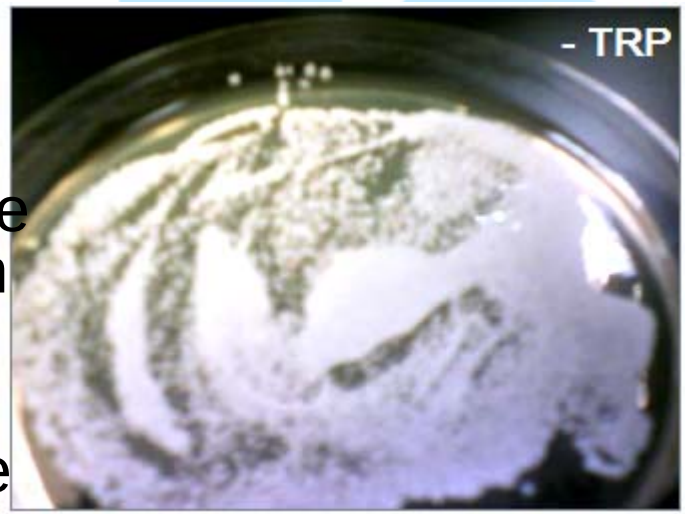


Table 1. Phenotypes in Negative Control - Water

Concentration	YPD	– TRP	– ISO
100 µl	12	85	0
200 µl	20	97	0
300 µl	23	125	0
400µl	18	112	0

Table 2. Phenotypes in Positive Control - EMS

Concentration	YPD	– TRP	– ISO
0.5%	65	120	0
1.0%	53	150	0
1.5%	37	210	1
2.0%	25	350	1

Table 3. Phenotypes on Different Media – Diet Pepsi® Post Mix Syrup

Concentration	YPD	– TRP	– ISO
50 µl	Lawn 153	Lawn 139	0
100 µl	Lawn 92	101	0
150 µl	Lawn 56	125	0
200µl	Lawn 88	73	0

Figure 1. *S. cerevisiae* colony growth in negative control

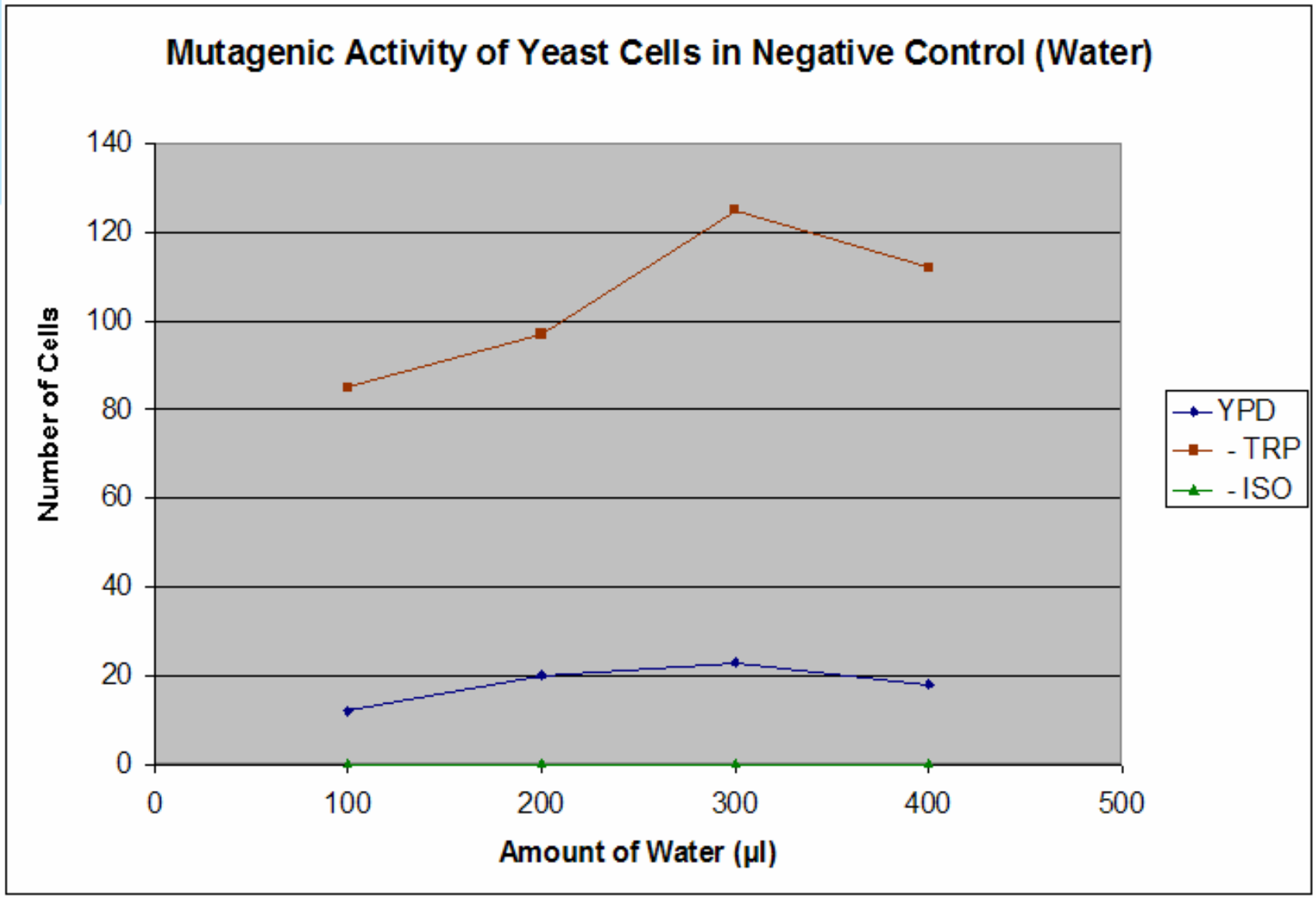


Figure 2. *S. cerevisiae* colony growth in positive control

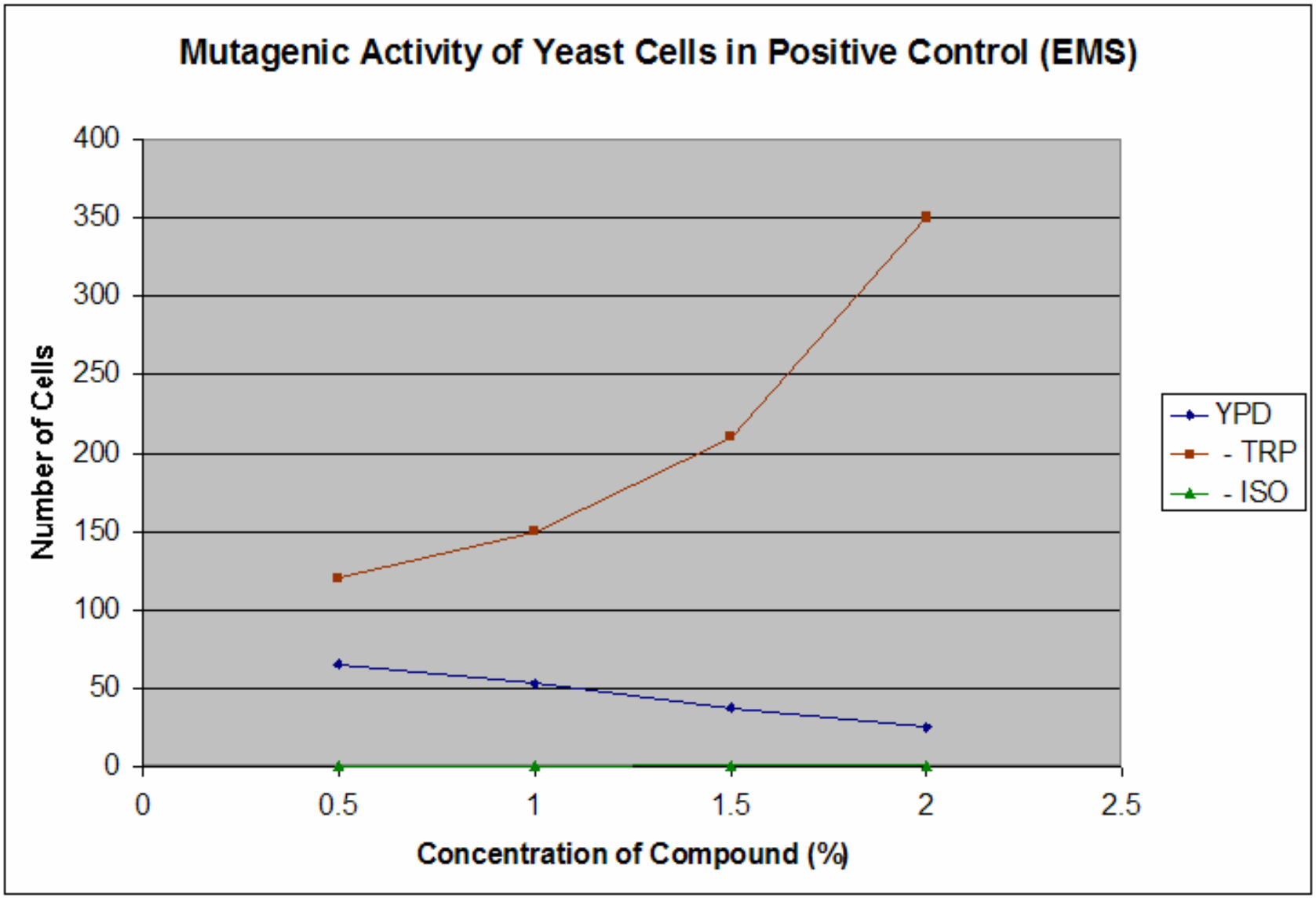
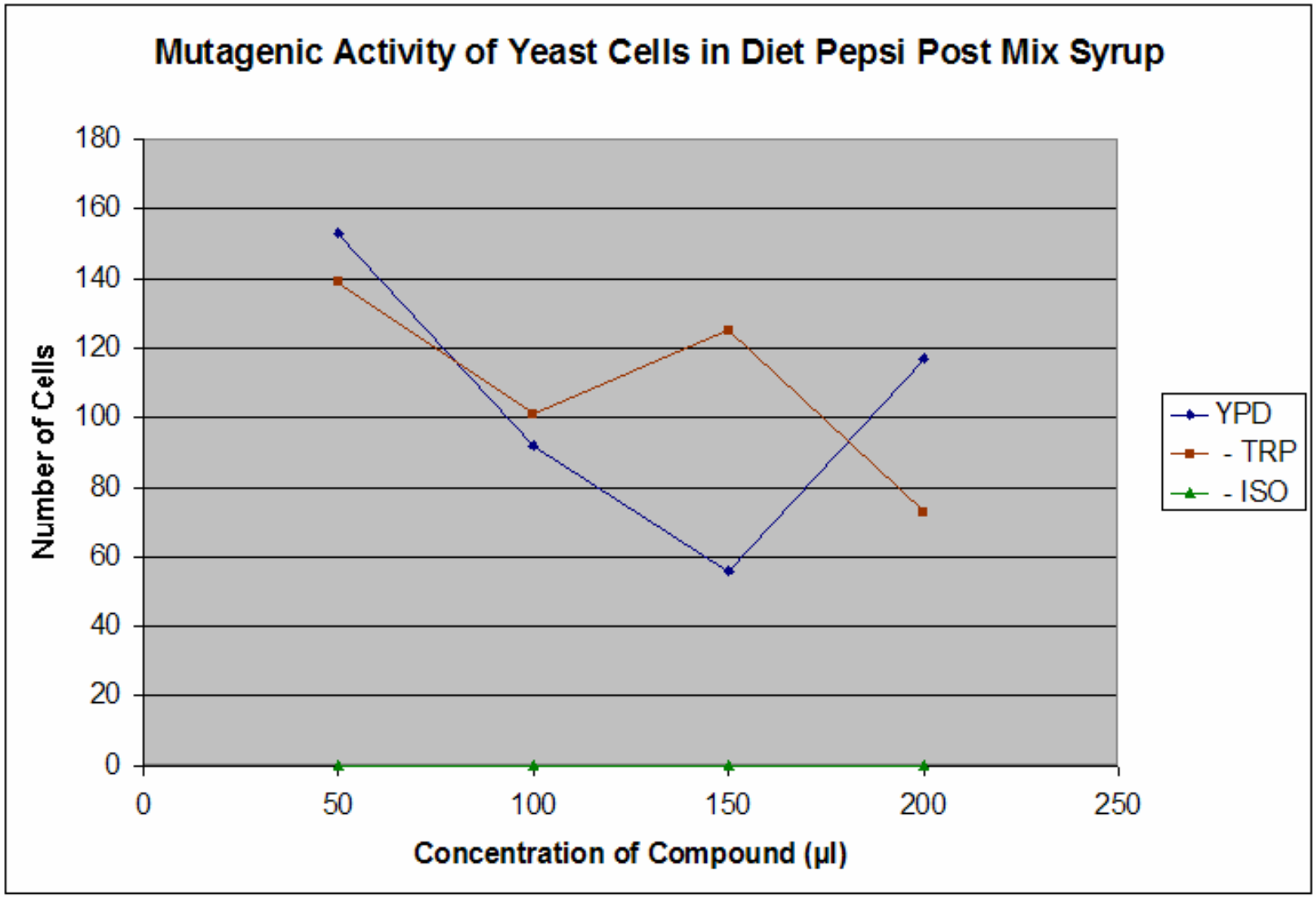


Figure 3. *S. cerevisiae* colony growth in test compound



Discussion

- The negative control had yeast cell growth in all four concentration on the YPD plates and the media plate lacking tryptophan. There were no growth in the media plate lacking isoleucine.
- The positive control had significantly more cell growth in both the YPD plate and the media plate lacking tryptophan compared to the negative control. There were two cell growth in the media plate lacking isoleucine.
- In the YPD media plates treated with the test compound, there were pink colonies amidst the lawn which indicates that the white colonies were mutated and the cells underwent mototic crossing over.
- In the media plates lacking tryptophan treated with the test compound, there were lawns and white colonies which indicates that the cells underwent mitotic gene conversion.
- In the media plates lacking isoleucine treated with the test compound, there were no growth indicating that there were no reverse point mutation.

- The hypothesis appears to be correct in which the presence of Diet Pepsi® post mix syrup mutated the *S. cerevisiae* colonies.
- The pink yeast colonies suggest that the cells were mutated and the number of colonies were greater than the number of colonies of the positive control.
- There were six molds growing on the YPD plate with 150 µl of the mutagen which may have been caused by contamination. There were significantly less colonies growing on the plate.
- The plates lacking tryptophan had similar results as the negative control. The number of colonies formed were not significantly different.
- In the plates lacking isoleucine, there were no colonies present. Most soft drinks have a pH of 3 to 4.5 and the acidity of the test compound may have prevented the colonies from forming.
- In the future, treating the plates with a smaller concentration may be easier to distinguish the different colonies formed.
- Choosing a compound that contains only aspartame can be used in the future to test if the aspartame in diet soda is the only factor that is carcinogenic.

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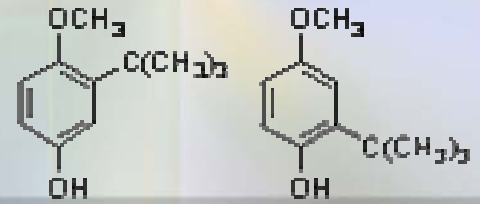
I determined that Kool-Aid is toxic!

Kool-Aid: The Facts

- Kool-Aid is an artificially flavored drink manufactured by Kraft Foods.
- Ingredients include, Citric Acid, Salt, Modified Cornstarch, Calcium Phosphate, Artificial Flavor, Ascorbic Acid, Artificial Color, Natural Flavor, and BHA.
- Upon further research of Kool-Aid, it was shown that the compound BHA (Butylated hydroxyanisole) might be a potential carcinogen/mutagen.
- BHA is found as a white/yellowish, waxy solid, and is used in foods to help preserve fats, or in compounds like Kool-Aid, in order to preserve freshness.
- BHA is an antioxidant, where Oxygen reacts with BHA rather than oxidizing fats, preventing foods from spoiling quickly (Kalus 1994).
- In this experiment, it was hypothesized that Kool-Aid contains components that are mutagenic and carcinogenic.
- Through research, it was determined that these mutagenic/ carcinogenic characteristics may be due to the presence of a freshness preserver called BHA, which is found in Kool-Aid (Kalus 1994).



Butylated hydroxyanisole
http://upload.wikimedia.org/wikipedia/en/8/8f/BHA2.png

Kool-Aid: The Hypotheses

- Kool-Aid is predicted to contain mutagenicity due to its ingredient BHA, which was researched to have potential mutagenicity.
- It is predicted that the experiment should show mutations through the presence of cell colony growth on various treatment plates, and that colony growth should increase as concentration increases. This shows that an increase in concentration of the treatment (Kool-Aid) also increases the number of mutations taking place.
- It is also predicted that there will be dense, lawn-like, white colonies found on the YPD plates, showing that there was normal colony growth.

Kool-Aid: The Experimental Materials/Methods

- By using 0.1 M potassium phosphate buffer, the Kool-Aid was first diluted to a fluid consistency.
- The buffer/Kool-Aid mixture was then centrifuged, and the aqueous top layer of Kool-Aid was transferred with a pipet in doses of 100 µl, 200 µl, 300 µl, and 400 µl.
- The Kool-Aid was added in increasing doses to help visualize whether an increase in concentration produces a higher mutagenic activity, whether there is no change in the rate of mutagenesis, or whether an increase in concentration tends to kill the yeast cells.
- The doses were added to separate tubes pre-filled with 100 µl of distilled water.
- The tubes were placed in a heat bath for 30 minutes and added in equal aliquots of 100 µl to each of the treatment plates, including the YPD plate, plates lacking Tryptophan, and plates lacking Isoleucine.
- The plates were incubated at 28ºC for a week, and observed for phenotypes/number of colonies.
- Positive and Negative controls were used to ensure an accurate and working experiment.
- A positive control was used to ensure a working assay. The positive control contained concentrations of EMS ranging from 0.5%, 1.0%, 1.5%, and 2.0%
- A negative control was used to ensure that no spontaneous mutations were occurring. This was done by using concentrations of water ranging from 100µl, 200µl, 300µl, and 400µl.

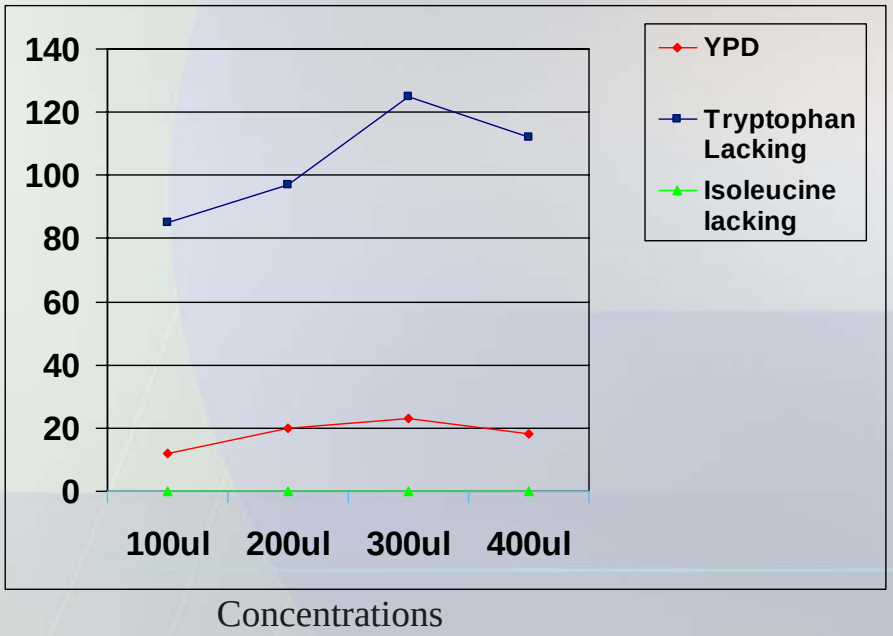
Kool-Aid: Control Results

Negative Control			
Concentrations	**YPD Plates**	**Tryptophan Lacking Plates**	**Isoleucine Lacking Plates**
100µl	12	85	0
200µl	20	97	0
300µl	23	125	0
400µl	18	112	0

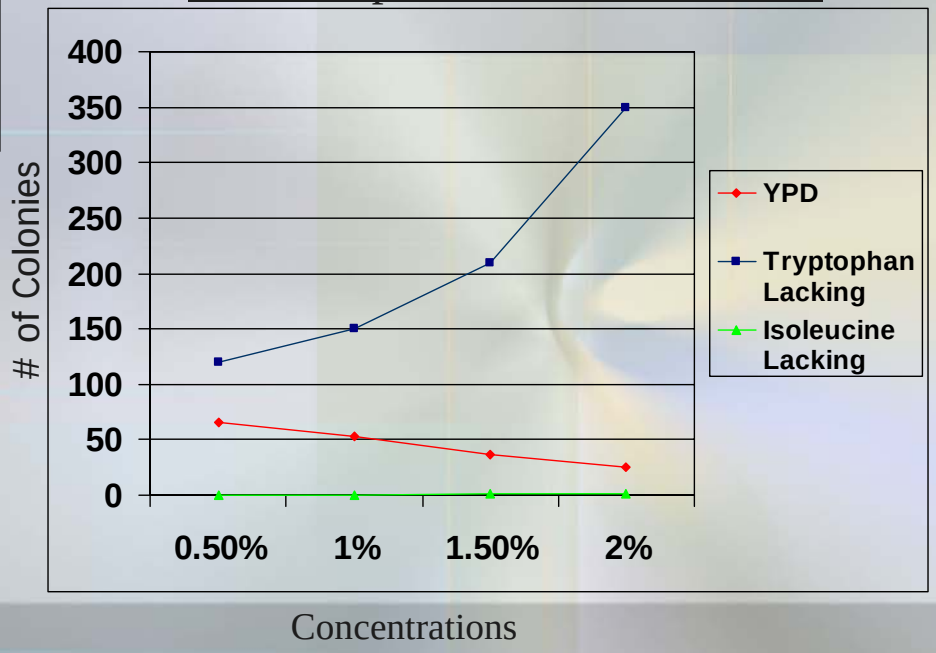
Positive Control			
Concentrations	**YPD Plates**	**Tryptophan Lacking Plates**	**Isoleucine Lacking Plates**
0.5%	65	120	0
1.0%	53	150	0
1.5%	37	210	1
2.0%	25	350	1

Kool-Aid: Control Results

Dose/Response: Negative Control

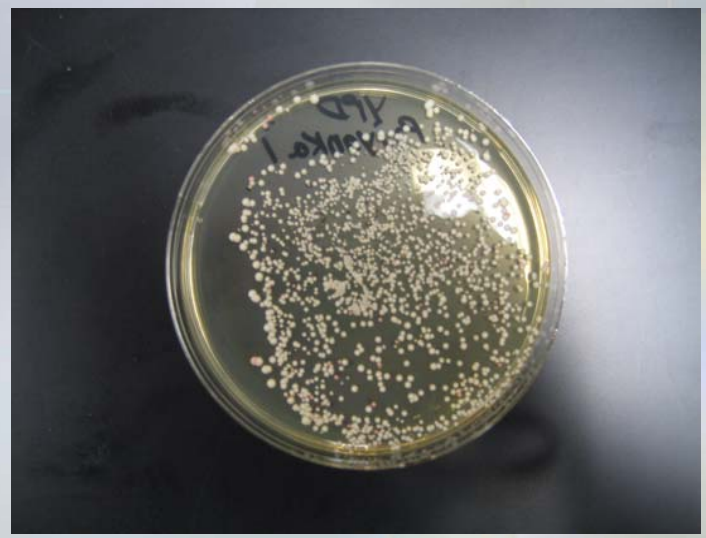


Dose/Response: Positive Control



Kool-Aid: Treatment Results

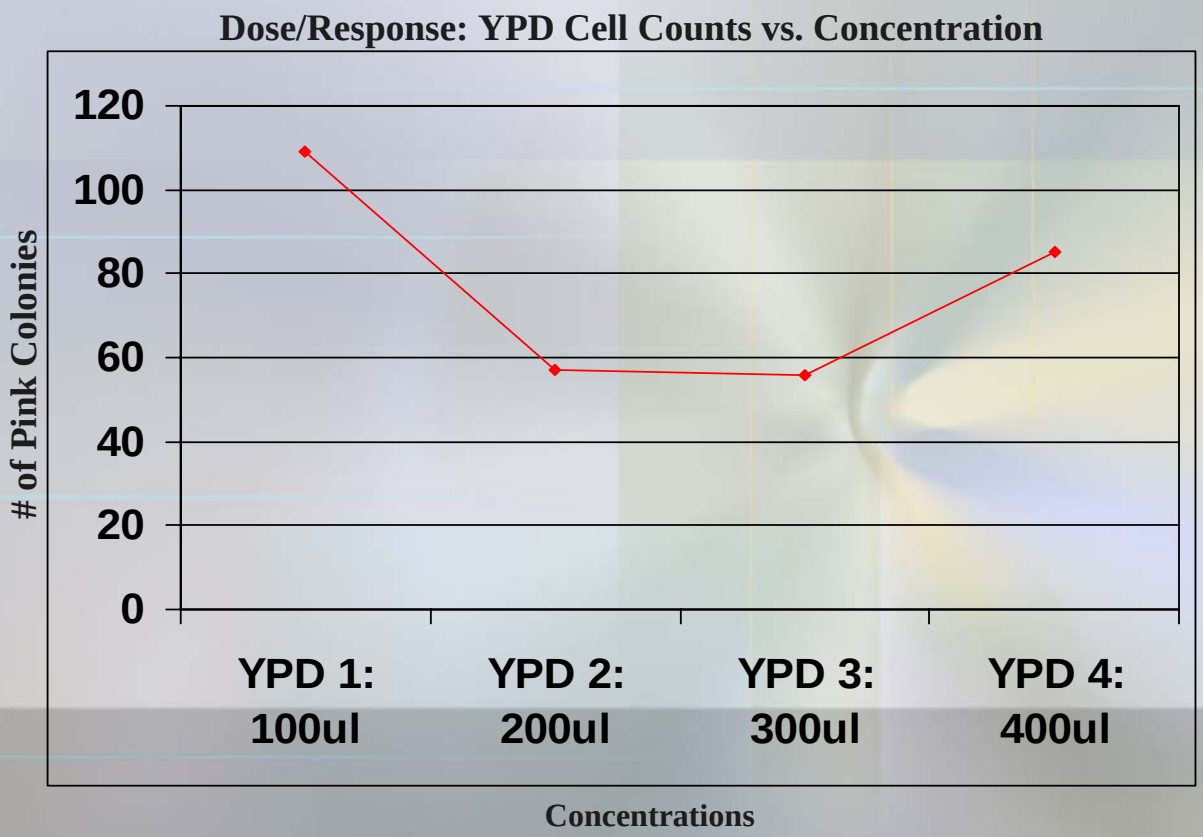
- The plate results showed that the YPD plates contained pink colonies.
- On the other hand, the plates that lacked Isoleucine, and plates that lacked Tryptophan did not have any colony growth.
- Overall, the YPD plates contained a significantly low number of white colonies than expected. Rather than a continuous and dense grass of white colonies, there were sparse white colonies, with various pink colonies within them.
- The number of pink colonies tended to decrease with an increase in concentration, until a concentration of 400µl was reached. At this point, the number of colonies began to increase.



The images shown above show the abnormally low number of yeast colonies that were found on the YPD plates. Rather than a dense lawn of colonies, there were sparse white colonies found with pink colonies embedded between them.

Kool-Aid: Treatment Results

YPD Cell Counts	
Plate	**Number of Pink Colonies**
YPD 1: 100µl concentration	109
YPD 2: 200µl concentration	57
YPD 3: 300µl concentration	56
YPD 4: 400µl concentration	85



Kool-Aid: Treatment Analysis

- Even though the YPD plates contained pink colonies, which represented the presence of Mitotic Crossing Over mutations (recombinational repair), the decrease in the number of cells with an increase in concentration showed that a chemical in Kool-Aid was causing toxicity to kill the yeast cells.
- As shown in the dose/response graph for the treatment, an increase in the concentration dramatically decreased the number of pink colonies found on the plate cultures. For example, an increase in the concentration from 100ul to 200ul caused a decrease in pink colony growth by approximately half.
- Oddly, at a concentration of 400ul, it was expected that the number of pink colonies decreased even further, but rather, the number of colonies increased. This may be due to experimental error, or a chemical reaction that occurred at higher concentrations. Further studies would need to be conducted to analyze the cause of these results.
- The theory that Kool-Aid was toxic was also supported by the lack of a white, dense lawn of cells found on the plate cultures, and rather, sparse and few, white colonies found.
- Lastly, there were no cells found on the plates lacking Tryptophan and those lacking Isoleucine, showing that there were no Mitotic Gene Conversion mutations or Reverse (point) mutations occurring.

Kool-Aid: Control Comparison Analysis

- Since the YPD treatment dose/response curve was greater than that of the negative control, it can be concluded that mutations were occurring, and that they were not related to merely spontaneous mutations (Zimmerman 1975).
- Since the YPD treatment dose/response curve was greater than that of the positive control, it can be concluded that the assay was working properly.
- Thus, the positive and negative controls served as a reference, and confirmed both a working experiment and an accurate experiment.

Kool-Aid: Hypotheses Analysis and Future Researches

- The hypotheses were confirmed in that there were pink colonies found on the YPD plates, which showed that there might have been mitotic crossing over mutations occurring.
- The number of pink colonies decreased with the concentration from 100µl, 200µl, and 300µl.
- Thus, though the presence of pink colonies on the YPD plates showed the occurrence of mutations, the decrease in the number of pink colonies due to an increase in concentration shows that the Kool-Aid was also toxic.
- The number of white colonies found on the YPD plates were significantly less than expected, showing that some chemical in the Kool-Aid sample was toxic to the yeast cells and killed them.
- In order to better serve the experiment, it would be necessary to use more concentrations of the Kool-Aid to see the effect of both lower and higher concentrations.
- It was also help to use smaller concentrations of the Kool-Aid, like 25-100ul rather than 100-400ul, in order to see more accurate effects of concentration on the cell count.
- Since the Kool-Aid contained several ingredients, it would be necessary to test each of the contents found in Kool-Aid in order to conclude which chemical caused the mutagenicity and/or toxicity.

Kool-Aid: An Application to our diet

- Once further studies have been conducted to determine which chemical found in Kool-Aid caused mutagenicity/toxicity, manufacturers can work to find healthier alternatives to this ingredient
- For example, if BHA is found to cause toxicity in Kool-Aid, due to it's oxidative ability to help preserve foods for a longer amount of time, it may be necessary to find an alternative method to sustain the preservation of foods.
- Consumers can also gain knowledge about potentially hazardous chemicals found in common foods, such as Kool-Aid, and it can then be their choice to consume or not to consume the product.

References

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