**Environmental Agent Effect on Microbes Marcus Stevens October 4, 2015**

1. Introduction

In this experiment, four different concentrations/exposures of an environmental agent were applied to two of the most studied microbes in the world, yeast and bacteria. This lab determines if there is any effect on the growth of the two microbes as a result of the different concentrations applied to the microbes. There are four test tubes per microbe, A, B, C, and D, tested in this experiment. Test tube D serves as the control, since it only contains 1000ul of SDF or sterile water and 100ul of microbe, no agent. The tested microbes are grown on a selected dish/plate contained within an incubator in order to analyze and record the quantity of colonies that developed in a certain span of time.

**Purpose:** The purpose of this experiment is to acquire dependable information on the effect of multiple concentrations/exposures of an environmental agent on two well-tested microbes, yeast and bacteria.

**Hypothesis:** When four different concentrations of an environmental agent are tested for the effect on the growth patterns of two microbes’, yeast and bacteria, the varying concentrations will have no effect on the growth of those microbes.

1. Materials and Methods (Procedure)

**Materials:**

**1.** One micropipette **2.** One vortex machine **3.** Sterile water/ sterile dilution fluid (SDF) **4.** Sterile pipette tips **5.** Sterile spreader bar **6.** Eight test tubes **7.** Six YEPD plates/dishes for the microbe/agent solution to grow **8.** Selected yeast and bacteria **9.** Incubator

**Procedure:**

1. Using a sterile tip on a micropipette, transfer 500uL of the agent into a test tube containing 8.9 mL of sterile dilution fluid (SDF). Transfer 500 uL of SDF or sterile water to the tube. Briefly vortex to mix.
2. Using a sterile tip on a micropipette, transfer 100uL of the agent into a test tube containing 8.9 mL of sterile dilution fluid (SDF). Transfer 900 uL of SDF or sterile water to the tube. Briefly vortex to mix.
3. Using a sterile tip on a micropipette, transfer 20uL of the agent into a test tube containing 8.9 mL of sterile dilution fluid (SDF). Transfer 980 uL of SDF or sterile water to the tube. Briefly vortex to mix.
4. Using a sterile tip on a micropipette, transfer 1000 uL of SDF or sterile water to the tube. Briefly vortex to mix. This serves as a control.
5. Using a sterile tip on a micropipette, transfer 100uL of yeast (or bacteria) suspension into the tubes from steps 1-3 above. Briefly vortex to mix.
6. Allow the tubes to sit for 15 minutes.
7. Using a sterile tip on a micropipette, transfer 100uL of yeast onto pre-labeled YEPD (or LB) plates. Spread with a sterile spreader bar.
8. Give the plates to your teacher or place upside down in an incubator.
9. After sufficient growth to visualize colonies (about 2 days), count the surviving colonies.
10. Graph the survivorship, x-axis = concentration of agent and y-axis = total colonies.
11. Results

**Data:**

|  |  |  |  |
| --- | --- | --- | --- |
| **A – 0% concentration** | **B - .1% concentration** | **C - 1% concentration** | **D - 10% concentration** |
| 8.9 ml sterile water (SDF) | 8.9 ml sterile water (SDF) | 8.9 ml sterile water (SDF) | 8.9 ml sterile water (SDF) |
| .1 ml yeast | .1 ml yeast | .1 ml yeast | .1 ml yeast |
| 1 ml sw | .99 ml sw | .9 ml sw | 0 ml sw |
| 0 ml toxicity agent | .01 toxicity agent | .1 ml toxicity agent | 1 ml toxicity agent |

**.1 ml from each test tube above was transferred onto a separate YEPD plate.**

|  |  |  |
| --- | --- | --- |
| **Exposure -** | **# of Yeast Colonies** | **# of Bacteria Colonies** |
| **A – 0%** | 2 colonies | 20 colonies |
| **B - .1%** | 30 colonies | 150 colonies |
| **C – 1%** | 110 colonies | 215 colonies |
| **D - 10%** | 120 colonies | 220 colonies |

**This is the data recorded for each microbe and each exposure.**

**Survivorship Graphing:**

1. Conclusion

It was originally thought that when four different concentrations of an environmental agent are tested for the effect on the growth patterns of two microbes’, yeast and bacteria, the varying concentrations will have no effect on the growth of those microbes. This hypothesis was clearly faulty as both the data table and survivorship graphs show. The data recorded explicitly presents a significant range between 0% concentration (the control), and 10% concentration. There was a large impact on the rate of growth in regard to the microbes’ colonies. As represented by the recorded data, the higher the concentration/exposure to the environmental agent, the lower the number of colonies. This trend was very noticeable in both graphs listed above.

Another trend that was observed is that the bacteria had a much larger count of colonies that formed in comparison with the yeast. The control concentration for the bacteria had approximately 100 more colonies than the control for the yeast. This might have happened because of the fact that yeast is a eukaryotic cell type, which implies that with all of its organelles and much larger chromosomes, it takes a longer time for the yeast to grow and develop than the bacteria.

A limitation that was detected for this experiment is the process of recording data. The procedure that was used to count the number of colonies was basically counting by hand. With every colony that was counted, a dot was made, with a sharpie marker, where the colony was on the labeled plate. Since not every colony could accurately be counted, simply because of human error, this is considered as a limitation. This could easily be fixed by either having multiple people counting the colonies for ensurence, or having a machine to count them. Another limitation that relates to this subject is that the procedure listed above does not show how to count the colonies. Therefore, the experiment cannot be repeated exactly how it was originally performed.