**CMU Lab #1 Marcus Stevens March 3, 2016**

1. Introduction

In this lab the common bacterium, Escherichia coli (E. coli), was used to the patterns of genes’ on and off signals. Lactose, a disaccharide made of glucose and galactose, was used as well. In order for E. coli to utilize the lactose, it must first metabolize it into two monomers. Since glucose is a monosaccharide, it can be directly used to generate energy. However, galactose must rearrange itself before it can be operable by the E. coli. This is where the enzymes are required. In order to assay the on and off signals associated with the breaking down of lactose, the quantity of ß-galactosidase, the enzyme responsible for the separation of lactose, were measured. However, since it is extremely hard to test galactose and glucose, ortho-Nitrophenyl-β-galactoside (ONPG) was used as a lactose substitute. ß-galactosidase separates the colorless ONPG into galactose and arthonitrophenol which gives off a yellow color. By measuring prominence of arthonitrophenol (yellow color) in cell extracts, grown under various conditions, quantitative data can be recorded for the amount of ß-galactosidase enzyme present in the cells. In all, this experiment demonstrates if the expression of lactose genes in E. coli can regulated by availability of sugars.

**Purpose:** In this lab, we will determine that in the absence of lactose, E. coli does not manufacture the enzymes required to catalyze the chemical reactions needed for utilization of lactose as an energy source, and vice versa.

**Hypothesis:** When ONPG/lactose and glucose are applied to E. coli, the prominence of ß-galactosidase will not be affected when it is incubated/grown in various conditions for certain amounts of time.

1. Materials and Methods (Procedure)

**Materials:** Refer to separate sheet

**Procedure:** Refer to separate sheet

1. Results

Specific Activity = Absorbance at 420 nm / Klett Spec Reading

Refer to separate page for data table

1. Conclusion

It was hypothesized that when ONPG/lactose and glucose are applied to E. coli, the prominence of ß-galactosidase will not be affected when it is incubated/grown in various conditions for certain amounts of time. This appears to be false according to the data. It shows that when lactose, or ONPG in this case, is added to E. coli bacteria, the activity of the enzyme responsible for the breaking down of the lactose is increased. This makes sense too. Since lactose is a potential energy source for the bacteria, it needs the ß-galactosidase enzyme to utilize its energy. Therefore, the enzyme will have more activity when lactose is present. However, the results appear to show that when both lactose and glucose are added to the E. coli bacteria, the activity decreases significantly in comparison to the lactose bacteria. This is because glucose is the sugar preferred by the E. coli, and if glucose is in supply, then the bacteria will potentially break down glucose over lactose (ONPG). Therefore, when glucose is present, enzyme activity is repressed to a certain degree.

There were several limitations that were in this experiment. One is the lack of accuracy in the recorded data. It had no distinct pattern, therefore, it could not accurately be interpreted. This is why the class data had to be used to interpret the outcomes correctly. Another limitation is the time of incubation in the procedure. This can be classified as a limitation because the times of incubation were not precisely followed, and they could have been over incubated.