Lab Report: Lac Operon Virtual Lab

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Materials & Methods (refer to the Student's Manual)
Qualitative Procedure (refer to the Student's Manual)
Quantitative Procedure (refer to the Student's Manual)

Results & Discussion

In the first 10 minutes of the qualitative analysis, all three culture tubes were clear and there was no visible change in color. After 20 minutes, the glucose tube was clear, the lactose tube was yellow, and the tube containing both glucose and lactose was faintly yellow. These colors stayed consistent throughout the remaining time (**Figure 1**).

In the quantitative analysis, Miller units were calculated using the following equation:

$$1 \text{ Miller unit} = 1000 \times \frac{(A_{420} - (1.75 \times A_{550})}{t \times v \times A_{600}}$$

Where t is the time elapsed since the start of the reaction, v is the volume (mL) of the culture used in the assay, A_{420} is the absorbance of the ONP, A_{550} is the correction factor for the light-scattering effects of bacterial cells, and A_{600} is the optical density of the original culture. The miller unit stays stable throughout the experiment because the equation takes the many variables that could invalidate the direct comparison of β-galactosidase activity into account. Using the equation above enables one to identify a direct and accurate comparison between the cultures.

This equation was used to calculate the β-galactosidase activity present in each of the three cultures and higher Miller units indicated higher β-galactosidase activity. 10 minutes after the reaction began, the enzymatic activity of the lactose culture was 111.1 Miller units, the enzymatic activity of the glucose culture was 1.4 Miller units, and the enzymatic activity of the glucose and lactose culture was 41.7 Miller units. 60 minutes after the reaction began, the enzymatic activity of the lactose culture was 112.8 Miller units, the enzymatic activity of the glucose culture was 1.7 Miller units, and the glucose and lactose culture was 36.1 Miller units (**Figure 2-3**). The quantitative data shows that the level of β-galactosidase activity in the glucose and lactose culture was consistently between that of the glucose culture and the lactose culture and that the lactose culture had the highest enzymatic activity, while the glucose culture had the lowest in terms of Miller units, which corresponds to the absorbance rates of A_{420} (**Figure 4**).

The E. coli in the lactose tube produced the most amount of β-galactosidase. Both the qualitative (**Figure 1**) and quantitative analysis (**Figure 2-4**) shows that the E. coli in the lactose tube produced the most amount of β-galactosidase. The qualitative analysis consisted of observations on how yellow each culture appeared. The darker the yellow shade, the more β-galactosidase has been produced. This is because when β-galactosidase is present, it cleaves the substrate ONPG to produce the yellow-colored OPG. Meanwhile, the quantitative experiment provided the numerical amounts of Miller units from each

culture. Miller units adjust the absorbency of the yellow ONP (A_{420}) to account for factors like the time elapsed in the experiment and the bacteria cells in the cultures to give an accurate representation of the level of β-galactosidase activity in the culture. The test tube with only lactose as the prominent shade of yellow had the highest Miller unit measurements, which both indicate the highest levels of β-galactosidase activity.

Results of the Qualitative Procedure			
Time from Start of Reaction	**Glucose**	**Glucose + Lactose**	**Lactose**
10 minutes	Clear	Clear	Clear
20 minutes	Clear	Very faint yellow	Faint yellow
30 minutes	Clear	Very faint yellow	Yellow
40 minutes	Clear	Very faint yellow	Yellow
50 minutes	Clear	Very faint yellow	Yellow
60 minutes	Clear	Very faint yellow	Yellow

Figure 1: Using the methods described above, the color of each test tube was recorded every ten minutes, which is depicted in the table above.

Results of the Quantitative Procedure			
Time Elapsed Since Start of Reaction	**Glucose A_{600} = 0.3**	**Glucose + Lactose A_{600} = 0.3**	**Lactose A_{600} = 0.3**
10 minutes	A_{420} = 0.7 Miller units = 1.4	A_{420} = 1.0 Miller units = 41.7	A_{420} = 1.5 Miller units = 111.1
20 minutes	A_{420} = 0.9 Miller units = 1.7	A_{420} = 1.5 Miller units = 43.4	A_{420} = 2.6 Miller units = 119.8
30 minutes	A_{420} = 1.3 Miller units = 1.2	A_{420} = 2.0 Miller units = 35.9	A_{420} = 3.9 Miller units = 123.8
40 minutes	A_{420} = 1.6 Miller units = 0.9	A_{420} = 2.7 Miller units = 39.1	A_{420} = 5.2 Miller units = 125.9
50 minutes	A_{420} = 2.2 Miller units = 2.8	A_{420} = 3.5 Miller units = 38.9	A_{420} = 6.3 Miller units = 116.7
60 minutes	A_{420} = 2.7 Miller units = 1.7	A_{420} = 4.2 Miller units = 36.5	A_{420} = 7.5 Miller units = 112.8

Figure 2: Using the methods described above, A_{420} absorbance and the miller units for each of the three test tubes was calculated every ten minutes, which is depicted in the table above.

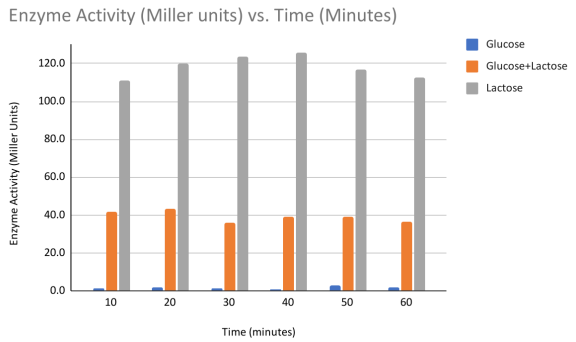

Figure 3: Using the methods described above, the enzymatic activity was calculated in miller units every ten minutes for each of the three test tubes, which is depicted in the graph above. Overall, the test tube containing glucose had significantly lower levels of enzyme activity in comparison to the test tubes containing lactose.

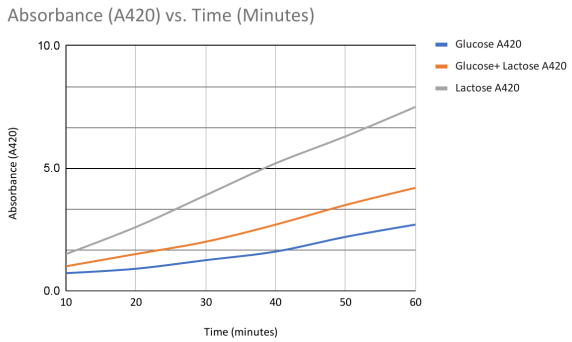

Figure 4: Using the methods described above, A_{420} absorbance was calculated every ten minutes for each of the three test tubes which are depicted in the line graph above. Overall, the test tube containing glucose had significantly lower levels of absorbance in comparison to the test tubes containing lactose.

Conclusion

The measurements taken both in the qualitative experiment and the quantitative experiment indicate that the tube containing only lactose exhibited more β-galactosidase activity than the other two tubes that contained glucose. Thus, the lac operon only produces the β-galactosidase when lactose is present and prefers the metabolization of glucose if possible, which exemplifies the E. coli cell's ability to conserve energy through gene regulation. When glucose is present, enzymes that metabolize lactose are not essential and therefore not produced by the cell, which is crucial in saving energy and resources.