Study on Catabolite Repression of the *E.coli* Lac Operon mechanism under IPTG induction and culture growth on Glucose and Glycerol carbon sources

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Abstract

This paper reports on the phenomenon of catabolite repression of the *E.coli* lac operon when induced by IPTG and grown simultaneously on Glucose and Glycerol mediums as carbon sources. The synthesis rate of the enzyme ß-galactosidase was measured for cultures growing in Glucose and Glycerol through monitoring of the catalysed reaction between the enzyme and the colourless compound ONPG, which results in the yellow compound ONP. Both cultures were also tested for growth rate by monitoring their optical density at 460nm at regular intervals. Analysis of the growth curves determined higher growth rate for Glucose cultures, and analysis of rate of enzyme synthesis per unit of cell mass determined higher production of galactosidase for Glycerol cultures, thus, clearly pointing Glucose as a better carbon source than Glycerol, for it maximized bacterial growth and minimized the production of secondary metabolites, such as galactosidase, for metabolization of alternative energy sources. The data also showed a clear direct correlation between cell growth and ß-galactosidase synthesis.

Keywords: Gene Regulation, Lac-Operon, ß -galactosidase, E.coli, Inducible system, catabolite Repression

1. Introduction

The *E.coli* lac operon is one of many regulatory mechanisms in bacteria that allows for fast adaptations in response to sudden changes of nutrient availability in their environment (Phillips et al, 2019). This mechanism is specifically directed at responding to the availability of Lactose in the environment by regulating the production of ß-galactosidase (encoded by the LacZ gene), lactose permease (encoded by the LacA gene), and galactosidase transacetylase (encoded by the LacY gene), all of which mediate lactose metabolism, as shown by Figure 1.

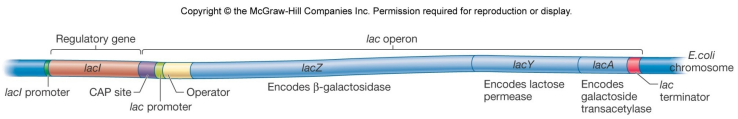

Fig.1. Visual representation on the structure of the *E coli* lac operon. Image retrieved from the lab manual (D'Souza & Gibon, 2020).

In the absence of lactose, the mechanism undergoes negative regulation, which consists of binding of a Lac repressor (encoded by the LacI gene) to the operator region, thereby preventing RNA polymerase from binding to the promoter site and transcribing the operon. According to the textbook "Molecular Biology: Principles and Practice" (Cox et at, 2015), a small quantity of LacZ and LacY molecules are still produced in order to induce lactose metabolism once Lactose becomes available despite the repressed operon.

In the presence of lactose, Allolactose is produced and acts as an inducer, binding to the Lac repressor and causing a conformational change which, in turn, causes for the repressor to dissociate from the operator and allows RNA polymerase to initiate transcription, allowing intermediate expression of the genes encoded by the operon. Isopropyl β-D-1 thiogalactopyranoside (IPTG) can also be used as an inducer, which differs from allolactose in the sense that *E.coli* do metabolize it as a carbon source, despite also being able to block the oppressor from binding the promoter. Both levels of control can be summarized by Figure 2.

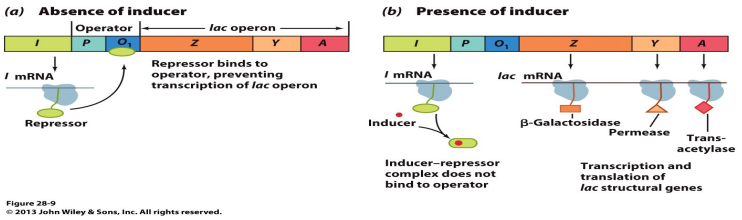

Fig.2. Visual representation on negative and positive regulation in the *E.coli* lac operon. Image retrieved from the lab manual (D'Souza & Gibon, 2020).

As indicated by the lab manual (D'Souza and Gibon, 2020), there is also a level of control relative to the quality of the energy source of the cell, in which a poor energy source causes for high levels of cAMP, causing it to bind the catabolite gene activator protein (CAP); this newly formed complex then bind to the CAP site in the lac operon and changes the conformation of the promoter, thus facilitating the binding of RNA polymerase, as well transcription of the encoded genes.

In the case of a good energy source, the levels of ATP production surpass the levels of AMP production and, Consequently, the levels of cAMP will be not be enough for the formation of the cAMP-CAP complex. This level of control is termed

is termed catabolite repression.

In the experimental system performed at the laboratory, the production of ß-galactosidase was monitored in two carbon sources (Glycerol and Glucose) through observation of *Escherichia coli* log phase growth. This was done by first determining the growth rate of the *E.coli* cell suspension using values obtained at regular intervals from an optical density test at 460nm.

The second part of the procedure consisted of determining the rate of ß-galactosidase synthesis before and after induction of the inducer IPTG through assaying, also at regular intervals, for color change in the culture once the reaction catalysed by this enzyme results into a colour change from colourless to yellow, as shown by Figure 3. The colour change was monitored by recording the sample's A_{420} values.

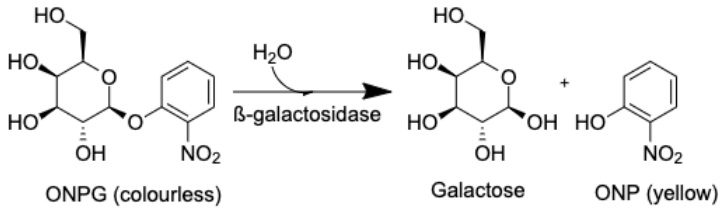

Fig.3. Mechanism of the catalytic reaction between ONPG and ß-galactosidase.

The two different sections of this procedure were performed simultaneously by separating the same culture into two (large and small culture), where the large culture was used as a means to determine the growth rate of the *E.coli*, and the small culture was used as a means to assay for the synthesis of ß-galactosidase.

Given the information above, the main purpose of the experiment was to obtain data on the growth rate of the large culture, as well as to use the assay from the small culture to determine the rate of ß-galactosidase per OD_{460} unit of cell mass synthesis in each of the carbon sources, as a means of discussing catabolite repression, as indicated by the lab protocol (D'Souza and Gibon, 2020).

The hypothesis for the results of the experiment was of a faster growth rate in the culture growing on Glucose than in the culture growing on Glycerol, since Glucose is a better carbon source. For this same reason, it was also expected that ß-galactosidase activity would be lower in the Glucose culture, once a better carbon/energy source should result in the phenomenon of catabolite repression.

2. Materials and Methods

The Experiment was carried on without modifications in accordance with the laboratory manual (D'Souza & Gibon, 2020).

Having performed the experiment, the calculations made were to determine units of β-galactosidase activity in each assay. This was done by obtaining the concentration of ONP from each sample through the Lambert-Beer Law (shown in equation I), as indicated by the laboratory manual (D'Souza & Gibon, 2020).

$$C = \frac{A}{\varepsilon l}$$

Where A is the absorbance A_{420} measured for each sample, ε is the molar extinction coefficient for ONP (equal to $4800 M^{-1}cm^{-1}$ according to the laboratory manual), and C refers to the concentration of ONP. The other calculations made in order to obtain the results did not have a set formula, rather, it was either composed of a series of unit conversions or were estimated from the plots; more information on the plot value estimation and the unit conversions can be found in detail at the results section of this report.

3. Results

The First part of the data analysis section consisted of building growth curves for the cultures in each carbon source, as shown by Figures 4.

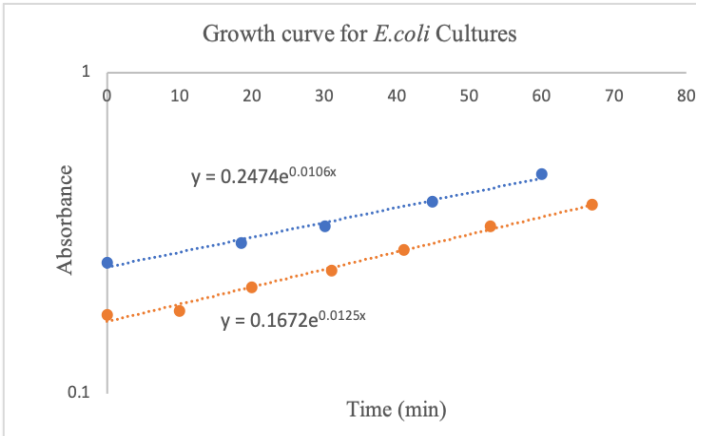

Fig.4. plot of Growth curves for the *E.coli* cultures in both carbon sources. The orange colour is related to the culture growing with Glycerol, and the blue colour is related to the culture growing with Glucose as the carbon source. An exponential trendline was added to both curves, and the y-axis was transformed into a logarithmic scale.

The plot was then used to estimate the doubling time of 0.908 (est. 68 min) and 1.082 (est. 57 min) for the Glycerol and Glucose cultures, respectively. In addition, the OD_{460} of the cultures at the sampled times for the assays was calculated based on the plot. The calculated values can be found at Tables 1 and 2 below.

Table 1. *Calculated OD_{460} values for Glucose culture at the times of β-gal assay sampling*

Clock Time	Sampled Time (min)	OD_{460}
0	27	0.329
2	29	0.336
4	31	0.343
6	35	0.358
8	39	0.374
12	43	0.390
16	47	0.407
20	51	0.424
24	55	0.443
28	59	0.462
32	63	0.482

Table 2. *Calculated OD_{460} values for Glycerol culture at the times of β-gal assay sampling*

Clock Time	Sampled Time (min)	OD_{460}
0	36	0.262
2	38	0.268
4	40	0.275
6	42	0.282
8	46	0.297
12	50	0.312
16	54	0.328
20	58	0.345
24	62	0.362
28	66	0.381
32	70	0.401

The second part of the data analysis section was focused in using the previous data to determine the rate of ß-galactosidase production in each of the cultures. This was done by measuring enzyme activity, first obtaining a value for concentration (C) of ONP through the Lambert-Beer Law (Equation 1). This value was then converted from M to nmoles/mL through multiplying the obtained value by $1x10^6$, given the conversion ratio from M to nmoles of $1:10^6$.

Given that each assay had a quantity of 2.8mL, the previous result was then multiplied the latter in order to determine the value of nmoles of ONP per assay, which was converted to nmoles per min, representing the Uβ-gal activity per 100µl of sample. In order to determine the Uβ-gal activity per mL of sample, the previously obtained value was multiplied by 0.1. The values obtained allowed for a plot of Uβ-galactosidase Activity vs. Time (Figure 5), from which the increase in Uβ-galactosidase was estimated (ΔEnzyme/ml). All these values were tabulated as shown by Tables 3 and 4.

Table 3. *Calculated values for Glucose E.coli cell culture*

nmoles	nmoles/min	Uβ-gal/ml	ΔEnzyme/ml	Time (min)
0.583	0.029	0.292	0.292	27
1.167	0.058	0.583	0.000	29
1.167	0.058	0.583	0.000	31
1.167	0.058	0.583	14.583	35
30.333	1.517	15.167	24.500	39
79.333	3.967	39.667	23.917	43
127.167	6.358	63.583	25.083	47
177.333	8.867	88.667	30.042	51
237.417	11.871	118.708	26.542	55
290.500	14.525	145.250	9.042	59
308.583	15.429	154.292	n/a	63

Table 4. *Calculated values for Glycerol E.coli cell culture*

nmoles	nmoles/min	Uβ-gal/ml	ΔEnzyme/ml	Time (min)
0.000	0.000	0.000	0.000	36
0.000	0.000	0.000	0.000	38
0.000	0.000	0.000	7.875	40
15.750	0.788	7.875	9.625	42
35.000	1.750	17.500	19.833	46
74.667	3.733	37.333	18.958	50
112.583	5.629	56.292	0.292	54
113.167	5.658	56.583	15.167	58
143.500	7.175	71.750	22.167	62
187.833	9.392	93.917	21.875	66
231.583	11.579	115.792	n/a	70

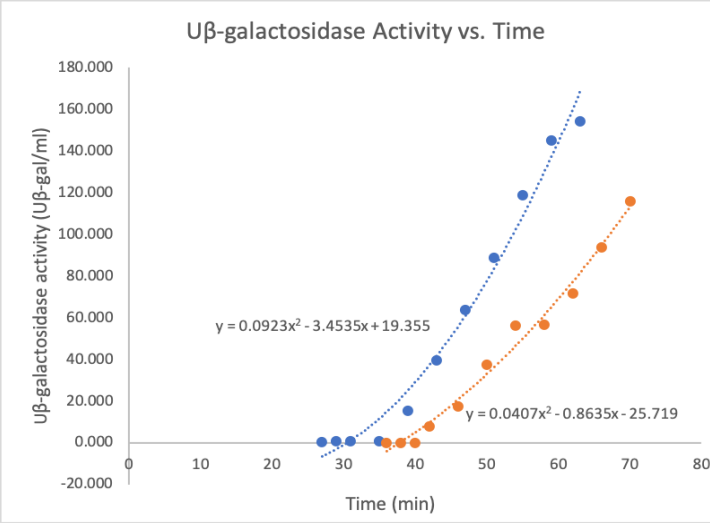

Fig.5. Plot of Uβ-galactosidase Activity vs. time. The orange colour is related to the culture growing with Glycerol, and the blue colour is related to the culture growing with Glucose as the carbon source. A polynomial trendline was added to both curves.

Based on the increase in enzyme content per mL, the OD_{460} values for each sampling time were determined, along with the values for β-gal synthesis rate, tabulated in Tables 5 and 6.

The final step of the data analysis was to determine the average rate of β-galactosidase synthesis before and after induction for each of the cultures, as indicated in Tables 7 and 8. For this experiment, the three first sampling times were considered as made before induction, and the remaining values were considered to be after induction.

Table 5. *Calculated* OD_{460} and β-gal synthesis rate *values for Glucose E.coli cell culture*

OD_{460}	β-gal synthesis rate (U/min/OD_{460})
0.329	0.221
0.336	0.000
0.344	0.000
0.359	10.169
0.374	16.375
0.390	15.321
0.407	15.401
0.425	17.680
0.443	14.972
0.462	4.889

Table 6. *Calculated* OD_{460} and β-gal synthesis rate *values for Glycerol E.coli cell culture*

OD_{460}	β-gal synthesis rate (U/min/OD_{460})
0.262	0.000
0.269	0.000
0.276	11.201
0.283	13.352
0.297	26.833
0.312	24.398
0.328	0.357
0.345	17.661
0.363	24.554
0.382	23.049

Table 7. *Calculated average β-gal rates for Glucose E.coli cell culture. The First three sampling times are representative of the period before induction, and the remaining sampling times are representative of the period after induction*

Time (min)	Average β-gal rate
0.262	
0.269	0.0738
0.276	
0.283	
0.297	
0.312	
0.328	13.543
0.345	
0.363	
0.382	

Table 8. *Calculated average β-gal rates for Glycerol E.coli cell culture. The First three sampling times are representative of the period before induction, and the remaining sampling times are representative of the period after induction*

Time (min)	Average β-gal rate
0.262	
0.269	3.733
0.276	
0.283	
0.297	
0.312	
0.328	18.601
0.345	
0.363	
0.382	

4. Discussion

According to the first part of the data analysis, the growth rate is much higher in the cultures growing on Glucose than in the cultures growing on Glycerol, with doubling times of 1.082 and 0.908 respectively.

The clearly higher doubling time for the Glucose cultures indicates a tendency for the confirmation of the hypothesis that Glucose is, indeed, a better carbon source than Glycerol, once it is logically reasonable to assume that the better energy source is the one to provide the culture more energy to grow, while the poor carbon source would do the contrary, thus forcing the organisms to induce the production of secondary metabolites.

In addition to the plot, Tables 1 and 2 also confirm higher growth rate for the Glucose culture by indicating higher cell density values for Glucose at every single one of the sampling times.

Given the higher number of cells present in the Glucose culture, it was logical that the plot in Figure 5 for β-galactosidase activity vs. time would indicate a higher amount of enzyme production for the Glucose culture, since more cells will produce more enzyme. If only this plot was taken into consideration, then it would have to be concluded that the second hypothesis for this experiment was not confirmed.

Nonetheless, if the rate of β-gal synthesis for both cultures is to be calculated per unit of cell mass/mL (as shown in Tables 5 and 6) instead of considering the entire culture, and averaged before and after induction (as shown in Tables 6 and 7), then it can be observed that on average, the glycerol culture has a higher rate of β-gal synthesis per cell, and the phenomenon of catabolite repression that happens upon poorer carbon sources such as Glycerol becomes clear, thus, confirming the second hypothesis for this experiment.

Taking into consideration that a good carbon source is defined as one that provides the organisms with enough energy to maximize growth and repress the use of other carbon sources through the phenomenon of catabolite repression (Bren et al, 2016), it can be concluded through the data from this experiment that Glucose is a better carbon source than Glycerol, for the data shows it was the source upon which the *E.coli* culture grew faster and synthesized less β-galactosidase per unit of cell mass.

Given the variables measured in this experiment, the use of a gratuitous inducer such as IPTG was absolutely vital, since using a normal inducer like allolactose would have caused it to be broken down and used as a carbon source, which in turn, would have interfered in the growth rates and synthesis of β-galactosidase. More specifically, it would have caused for higher growth rates in both cultures, as well as a possible change in regulation type of the lac operon, thus resulting in confusing β-gal synthesis rates.

The monitoring of enzyme synthesis was only possible due to the colour change in the reaction between ONP and β-galactosidase. In this reaction, shown by Figure 1, β-galactosidase reacts with the colourless compound ONPG and results in the yellow compound ONP. Given these conditions, the monitoring of ONPG concentration is directly related to β-galactosidase activity, since it is only produced as a result of a catalysed reaction between the enzyme and ONP.

For organisms whose environment is ever so rapidly changing like *E.coli* bacteria, it is fundamental to have control over the expression of catabolic genes, as to allow faster adaptation to those environments, as well as a method for conservation of energy and to ensure that only the necessary amount of protein is being produced (Adam et al, 2014).

In the case of the lac operon, it exists as a way for breakdown of alternative sources other than the primary source (Glucose) whose transcription is only necessary if the bacteria don't have access to their primary source.

The regulation mechanism of this operon then works to avoid the use of energy and space that takes place if the operon's transcription is turned on unless it is absolutely necessary for survival, which would be cases of absence of the primary energy source.

5. Acknowledgements

I thank Tobin and John for providing the data for the Glucose *E.coli* cell culture.

6. References

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7. Appendix

A. Raw data

Table A1. *Raw data for Absorbance vs. time of Glucose culture*

Time(min)	A
0	0.256
18.5	0.293
30.07	0.332
45	0.395
60	0.482

Table A2. *Raw data for Absorbance vs. time of Glucose culture*

Time(min)	A
0	0.175
10	0.181
20	0.214
31	0.242
41	0.279
53	0.331
67	0.386

B. Sample Calculations

Example Calculation of OD_{460} values for Glucose culture:

$$y = 0.2474e^{0.0106x}$$
$$y = 0.2474e^{0.0106*27}$$
$$y = 0.329$$

Example Calculation for Concentration (C) of ONP released in the sample for Glucose culture:

$$C = \frac{A}{\varepsilon l}$$

$$C = \frac{0.01}{(4800 M^{-1}\, cm^{-1}) * 1}$$
$$C = \frac{0.01}{(4800 M^{-1}\, cm^{-1}) * 1}$$
$$C = 2.083x10^{-7}\text{M}$$

Conversion of Concentration (C) value from M to nmoles/mL for the Glucose culture:

$$C = 2.083x10^{-7} M * 1x10^{6}$$
$$C = 0.2083\ nmoles/mL$$

Sample calculation for nmoles of ONP per assay:

$$[ONP] = C * 2.8mL\ of\ ONP\ per\ assay$$
$$[ONP] = 0.2083 \frac{nmoles}{mL} * 2.8mL\ of\ ONP\ per\ assay$$
$$[ONP] = 0.583\ nmoles$$

Calculation for units of β-gal produced per 100µL of sample:

$$\frac{\beta gal}{100}\mu L = {[ONP]}/{20minutes}$$

$$\frac{\beta gal}{100}\mu L = {0.583nmoles}/{20minutes}$$

$$\frac{\beta gal}{100}\mu L = 0.029\ nmoles/min$$

Calculation of production of units of β-gal per 1 mL of sample:

$$\frac{U\beta gal}{ml} = \frac{\beta\text{gal}}{100}\mu\text{L} * 0.1$$

$$\frac{U\beta gal}{ml} = 0.029\text{nmoles/min} * 0.1$$

$$\frac{U\beta gal}{ml} = 0.292$$

Calculation increase in enzyme content of the cultures per 4-minute period:

$$\Delta\frac{Enzyme}{ml} = \frac{U\beta gal}{ml}(at\ time\ 29) - \frac{U\beta gal}{ml}(at\ time\ 27)$$

$$\Delta\frac{Enzyme}{ml} = 0.583 - 0.292$$

$$\Delta\frac{Enzyme}{ml} = 0.291$$

Calculation of β-gal synthesis rate per OD_{460} unit of cell mass for each assay sampling time:

$$Synthesis\ Rate = \frac{\Delta\frac{Enzyme}{ml}}{4\ minutes * \text{OD}_{460}}$$

$$Synthesis\ Rate = \frac{0.292}{4\ minutes * 0.329}$$

$$Synthesis\ Rate = 0.221\frac{\text{U} * \text{OD}_{460}}{\text{min}}$$

Calculation for average rate of β-gal synthesis rate per OD_{460} unit of cell mass before and after induction:

$$Before\ Induction\ avg = \frac{0.221 + 0 + 0}{3}$$

$$Before\ Induction\ avg = 0.0738$$

$$After\ Induction\ avg = \frac{10.16 + 16.37 + 15.32 + 15.40 + 17.68 + 14.97 + 4.88}{7}$$

$$after\ Induction\ avg = 13.5438$$