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The Role of Glycerol and Isopropyl Thiogalactoside in *Escherichia coli* Growth and Lactose Induction of β-Galactosidase

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Enzyme induction was studied by performing β-galactosidase activity assay on a culture of *Escherichia coli* K-12 wild-type strain B23 induced by IPTG in the presence and absence of lactose. Our findings suggest that lactose-only media supported a slower growth than glycerol-containing media did. Furthermore, lactose was found to have no effect on *Escherichia coli* growth in glycerol, whereas its analogue, isopropyl thiogalactoside, negatively influenced growth. In terms of β-galactosidase activity, lactose and isopropyl thiogalactoside were also found to exert different effects. Lactose in the absence of IPTG appeared to be the stronger inducer, yielding a higher enzyme activity. Interestingly, the addition of both lactose and isopropyl thiogalactoside decreased enzyme activity to a minimal level.

The enteric bacterium *Escherichia coli* is capable of growing on a broad range of carbon sources. The composition of its nutrient environment is a prime determinant of what catabolic enzymes are synthesized by this organism. β-galactosidase is an enzyme that cleaves the disaccharide lactose into glucose and galactose. Its expression is induced by the presence of lactose, or its analogue, isopropyl thiogalactoside (IPTG). Substrates that support fast growth – such as glucose – have been shown to induce the lowest levels of β-galactosidase (2). Conversely, growth on a carbon source that supports slower growth than glucose results in a higher level of β-galactosidase (2).

Recent studies (3) show that β-galactosidase activity is high in minimal medium supplemented solely with glycerol and IPTG. Interestingly, it was found that β-galactosidase induction abruptly decreases and remains suppressed when lactose is added to glycerol culture. This observation is counterintuitive, as one would expect higher β-galactosidase activity in the presence of excess lactose. This leads us to suspect that the presence of glycerol may have an adverse effect on β-galactosidase induction by lactose and IPTG.

This study aims to understand the reasons for this observation by comparing growth and β-galactosidase induction of *E. coli* grown in different conditions: glycerol only, lactose only, glycerol with lactose, glycerol with IPTG, and glycerol with IPTG and lactose.

MATERIALS AND METHODS

M9 media supplemented with 0.2% glycerol was inoculated with *E. coli* K-12 wild-type strain B23 (supplied by Dr. Ramey, UBC) and incubated overnight in a shaking water bath at 37°C. The overnight culture was diluted in fresh media to yield 0.4 $O.D._{460}$ after 3 additional hours of incubation at 37°C. The culture was then centrifuged in a Beckman Model J2-21, rotor JA-20 at 10,000 rpm for 10 minutes at room temperature. The cells were washed with M9 at 10,000 rpm for 5 minutes at room temperature. The pellet was then resuspended in M9. The culture was further diluted to 0.2 $O.D._{460}$ and transferred to five flasks containing M9 supplemented with different carbon sources. The composition of the five media types were: (1) M9 with 0.2% glycerol; (2) M9 with 0.1% lactose; (3) M9 with 0.2% glycerol and 0.1% lactose; (4) M9 with 0.2% glycerol and IPTG; and (5) M9 with 0.2% glycerol, IPTG and 0.1% lactose, where the lactose was added 15 minutes following IPTG induction.

Turbidity was measured with a Spectronic 20 at 460 nm on an expanding timeline; and β-galactosidase induction was measured by ONPG assay (3).

RESULTS

Figure 1 delineates the growth rate of *E. coli* under the following growth conditions: (A) glycerol-only (B) lactose-only (C) glycerol-lactose, (D) glycerol-IPTG, and (E) glycerol-IPTG-lactose. We observed that both the glycerol-only and glycerol-lactose systems exhibited faster growth rates than the lactose system. This decrease in the growth rate of the lactose-only culture suggests that growth is slower in lactose than in glycerol or glycerol-lactose. This is expected from a culture that was previously growing on glycerol because it was not adapted to use lactose. The lactose-only culture had a concave growth curve, suggesting that the initial growth was slower than those of the other cultures and gradually increased during the course of the experiment. A concave growth curve was also seen

in the glycerol-IPTG culture. The growth rates for the remaining cultures were constant as expected, and as seen in previous experiment (3). It should also be noted that consistently higher turbidity reading was seen in the glycerol-IPTG-lactose culture. This was likely due to experimental error. Thus data was adjusted prior to graphical analysis.

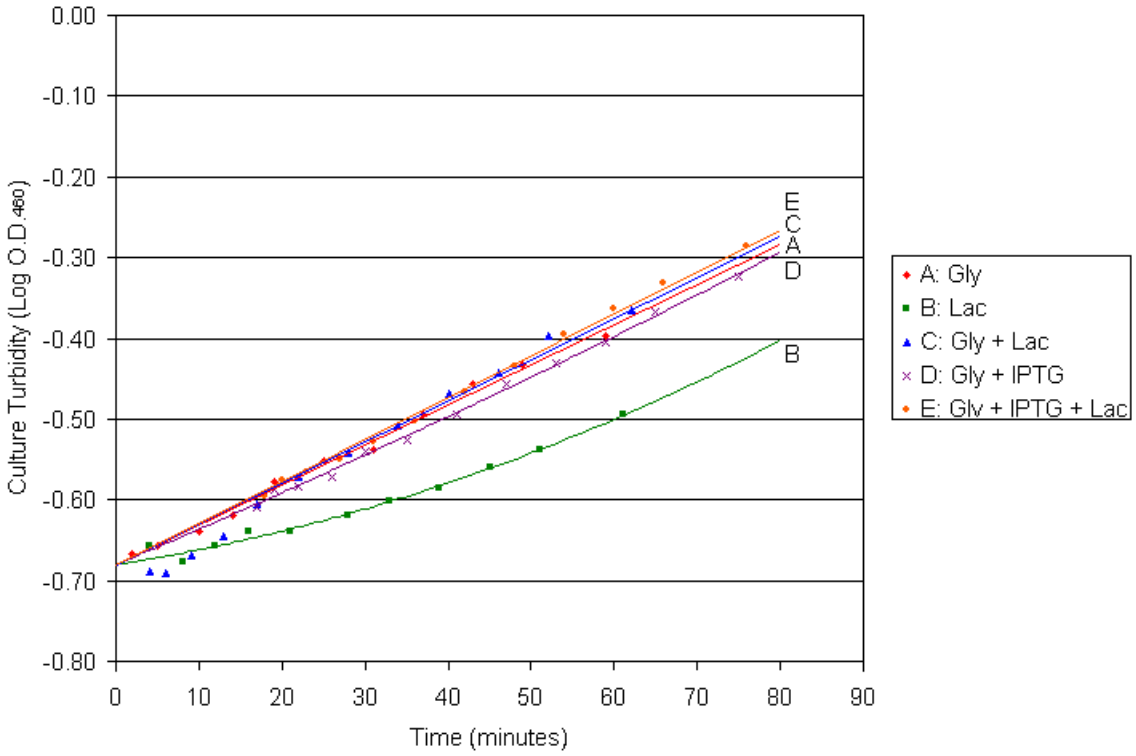

Figure 1. *E. coli* growth in the presence of different combinations of carbohydrates and β-galactosidase inducer. Turbidity of the cultures was measured at $O.D._{460}$. For cultures containing IPTG (D and E), turbidity readings commenced 15 minutes later, at which time lactose was added to E.

In β-galactosidase enzyme induction assay (Figure 2), each condition produced a unique trend. It should first be noted that the absence of a glycerol-only curve is due to undetectable enzyme activity. During the initial 15 minutes, the glycerol-IPTG culture and glycerol-IPTG-lactose culture had similar experimental conditions and were expected to produce similar curves. However, this was not seen in our experiment, possibly due to initial turbidity differences. Furthermore, enzyme activity measurements of the glycerol-IPTG culture produced erratic data. Thus, the curves on Figure 2 of these cultures did not begin with the same pattern. For the same reason, the result obtained from glycerol-IPTG culture will be less reliable for analysis of the data. Repeating the experiment would solve this problem.

The initial rates of increase in enzyme activity of lactose-only, glycerol-lactose and glycerol-IPTG-lactose were fast with respect to the glycerol-IPTG condition. As stated above, we suspect that the glycerol-IPTG culture had slower increase in enzyme activity due to turbidity difference. The other rates begin to diverge after 15 minutes. The glycerol-lactose condition produced a hyperbolic curve. The hyperbolic nature of the curve implies that the initial enzyme activity was fast but gradually slowed down over the course of the experiment. Enzyme activity in lactose-only condition also slowed down after 20 minutes and its curve showed the lowest rate of increase towards the end of the experiment.

The most interesting pattern of β-galactosidase activity can be seen in the condition containing glycerol, IPTG and lactose. The graph shows a steady increase in initial enzyme activity, induced by IPTG in the absence of lactose. At 15 minutes, upon addition of lactose, β-galactosidase activity dramatically slowed down until 40 minutes, at which time it began to increase again. The enzyme activity had a lag period after lactose was added, but accelerated again and eventually reached a similar level to the glycerol-lactose culture.

Because of the differences in growth in Figure 1, it was necessary to compare the relative enzyme activity among the conditions by taking into consideration the turbidity differences in the test samples. This can be shown by

specific enzyme activity of the four different conditions (Figure 3). At around 40 minutes, the lactose-only culture had the highest level of enzyme activity, followed by the glycerol-lactose culture, the glycerol-IPTG culture, and lastly the glycerol-IPTG-lactose culture. β-galactosidase induction increased steadily in the lactose-only culture and peaked at 40 minutes, after which induction decreased. The opposite is true for all the other glycerol-containing cultures. In these cultures, β-galactosidase activities decreased for the first 30-40 minutes, and then the activities climbed up as time progressed. The change in the glycerol-lactose culture was not as significant as others, as the enzyme activity between 15 to 50 minutes remained relatively constant. As expected, specific activities in the glycerol-IPTG culture and the glycerol-IPTG-lactose culture were similar in the first 15 minutes, during which the experimental conditions were identical. Upon addition of lactose, β-galactosidase activity in glycerol-IPTG-lactose dramatically decreased to minimal level and did not increase until 35 minutes into the experiment.

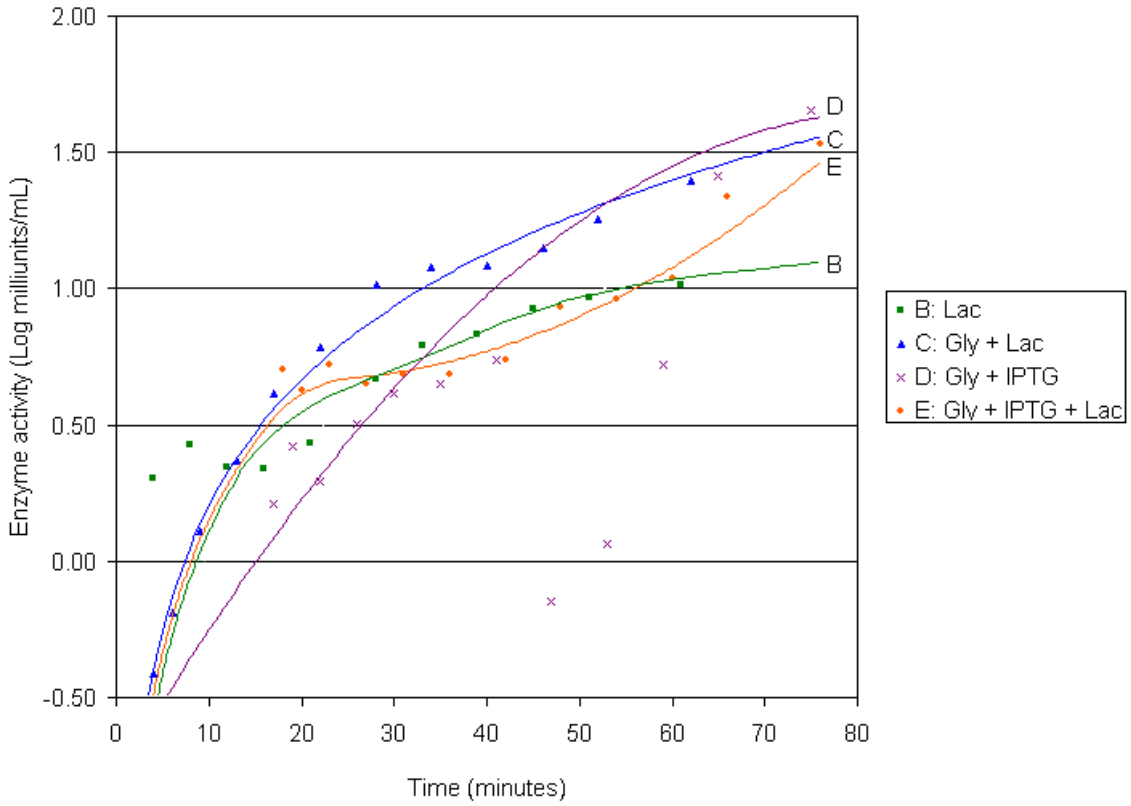

Figure 2. β-galactosidase induction in different combinations of carbohydrates and inducer. β-galactosidase activity under condition A was negligible and for this reason is not represented in the figure.

Despite the fact that the lactose-only culture had the highest β-galactosidase activity at forty minutes, induction seemed to have stopped and its enzyme activity level was superseded by those in glycerol-containing cultures by the end of the experiment. Both the glycerol-IPTG culture and the glycerol-IPTG-lactose culture showed remarkable increase in specific β-galactosidase activity towards the end of our experimental period.

DISCUSSION

The similar growth rates exhibited among *E. coli* cultures containing glycerol imply that glycerol was present in sufficient quantity to sustain growth. Additional carbon sources appeared to have no effect on growth when glycerol was present. The lactose-only culture showed a significantly lower growth rate than all the other glycerol-containing cultures. Since the machinery for glycerol metabolism was expressed in *E. coli*, no major adjustments were required by the cells when transferred from a glycerol-containing media to another glycerol-containing media. The lower growth rate of the lactose-only culture might have been due to the slow rate of lactose molecules entering the cell. Lactose molecules are brought into bacterial cells by LacY permease, whose expression is controlled by the *lac* operon. In the absence of an inducer, the *lacY* gene is expressed at a very low level. However, the concave

nature of the growth curve suggests that as the lactose metabolism system was induced by the lactose molecules, the growth rate increased. This is likely due to the extra energy produced from the glucose and galactose components of the disaccharide. Furthermore, the growth rate could increase at a higher rate once the permease was made in sufficient quantity to provide a surplus supply of lactose to the cell. The lag phase accounts for the adjustments that occur in *E. coli* when glycerol is replaced by lactose as the carbon source.

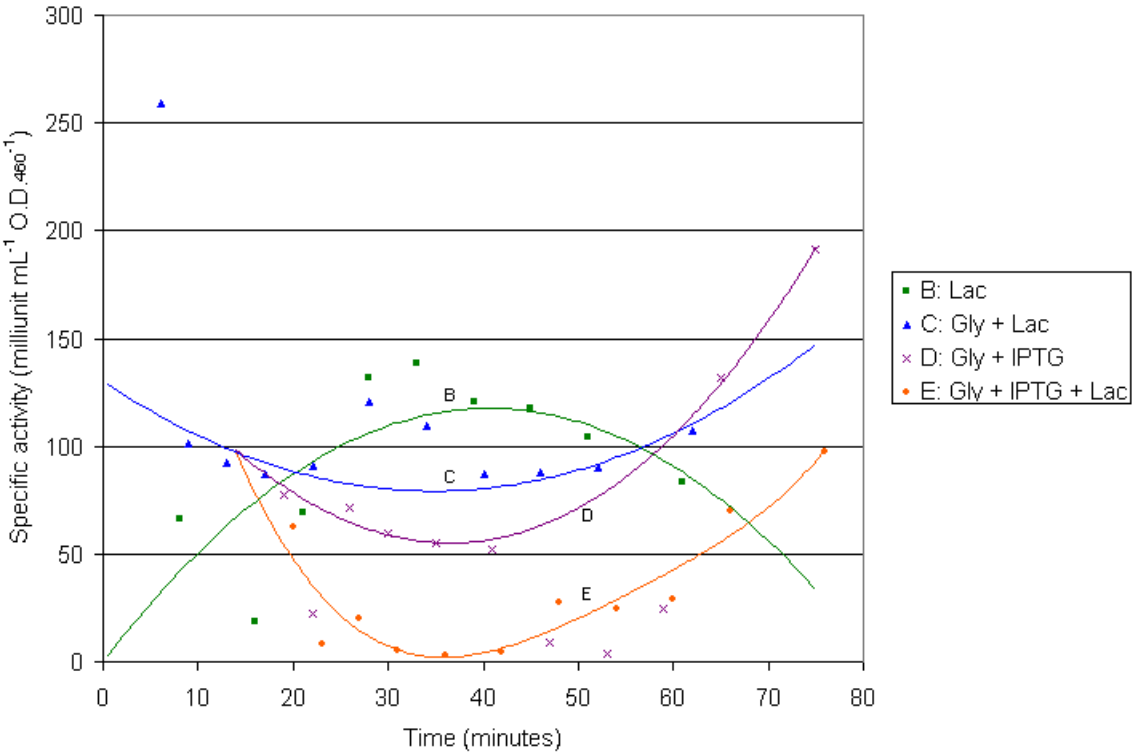

Figure 3. Specific activity of β-galactosidase in different combinations of carbohydrates and inducer. β-galactosidase activity is normalized for comparison among the different conditions.

Similar growth rates in glycerol-only and glycerol-lactose cultures show that lactose does not inhibit growth in glycerol. This may be an indication that cells were solely utilizing glycerol. Since glycerol was the original carbon source used to culture *E. coli*, one may suspect that there exists a correlation between the original carbon source and the growth pattern in our experiment. Another possibility may be that *E. coli* were utilizing glycerol because the induction of the *lac* operon was less efficient. This could be tested by monitoring the levels of glycerol and lactose remaining in the medium.

IPTG appears to have a negative effect on *E. coli* growth. IPTG is a lactose analogue that works by binding and inactivating the *lac* repressor. IPTG does not require any transport mechanism; rather, it can diffuse through the membrane, suggesting that the *lac* operon can be induced quicker by IPTG than by lactose. However, since IPTG is not cleaved by β-galactosidase to supply energy for the cell, expressing *lac*-related genes is expected to slow down culture growth rate. This may explain the slower growth rate of *E. coli* in glycerol-IPTG medium than in glycerol-lactose medium.

Due to the erratic nature of data from the β-galactosidase assay, inferences cannot be made from Figure 2 without giving misleading interpretations. However, one interesting observation did emerge. When lactose was added to the system containing glycerol and IPTG, there was a period where β-galactosidase induction momentarily halted. This resembles the findings from earlier experiments (1), which suggested that transient repression might be at play.

Despite the fact that the lactose-only culture had the slowest growth, Figure 3 shows that it had the highest enzyme activity. However, the effect was only temporary. This may be explained by the gradual decrease in available lactose, which can be confirmed by future experimentation by providing a continuous supply of lactose to the system. Catabolite repression of glucose produced from cleaving lactose may also contribute to the decrease in enzyme activity. In the remaining cultures, figure 3 shows that β-galactosidase activity was already induced before

measurements were taken. Plausible explanations are: 1) Centrifugation may change the behaviour of *E. coli*, 2) Time delay when transferring cells into new media after centrifugation. These are a few of the possible factors that may have contributed to the peculiar finding. Nonetheless, it is apparent that β-galactosidase activity increased when switched to lactose-only medium, whereas the opposite was observed in cultures with glycerol and lactose. This result supports our hypothesis that glycerol may exert a negative effect on β-galactosidase induction. When specific activities of β-galactosidase in glycerol-IPTG and glycerol-lactose were examined, it was surprising to see glycerol-lactose giving the higher activity. Since lactose requires a transport protein whereas IPTG does not, one might expect glycerol-IPTG to have the higher activity. This was not the case for unknown cause.

When the specific β-galactosidase activities of all three glycerol-containing cultures were examined, the system receiving both lactose and IPTG had the lowest activity. The addition of lactose actually reduced β-galactosidase induction rather than increased it. This decrease corresponded to the plateau of glycerol-IPTG-lactose system observed in Figure 2. Rather than having an additive effect on enzyme activity, it appears that inducers might need to be added at an optimal level in order to produce the desired result. In order to test whether this repression is non-specific to the type of inducer, but specific to the amount of inducer, experiments can be done using various combinations of differing amounts of inducers.

In summary, we conclude that lactose had no significant effect on *E. coli* growth in glycerol, whereas IPTG can cause a slight decrease in growth. Furthermore, lactose initially appears to be a stronger inducer than IPTG. Furthermore, the amount of inducers added to the system might be an important factor to consider when examining β-galactosidase activity.

ACKNOWLEDGEMENTS

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Appendix I. Sample Calculations (Example from Flask B).

Sample time (min)	Turbidity ($O.D._{460}$)	ONPG added (H:M:S)	Reaction stopped (H:M:S)	Elapsed time (min)	Absorbance (A_{420})
4	0.210	18:33:00	19:47:00	74.0	0.235
8	0.220	18:33:30	19:38:00	64.5	0.272

Enzyme activity (EA):

$$\frac{EA}{\text{mL of enzyme}} = A \times \frac{1}{t} \times \frac{10^6}{15{,}000} \times Nv \times \frac{1}{Ev}$$

Where:

1 milliunit = 1 nmole of product formed per minute

A = difference in absorbance at 420 nm in the enzyme and the corresponding blank assay after the elapsed time for the enzyme assay

t = time in minutes for the observed change in absorbance

15,000 = the molar extinction coefficient for o-nitrophenol in the Spectronic 20 tube

10^6 = a correction to change the molar part of the extinction coefficient from mole per liter to nmole per milliliter

Nv = total assay volume (mL) at the time of the absorbance reading

Ev = volume of enzyme sample (mL) in the assay

$$EA_4 = 0.235 \times \frac{1}{74.0} \times \frac{10^6}{15{,}000} \times 3.8 \times \frac{1}{0.4}$$

$$= 2.01$$

$$EA_8 = 0.272 \times \frac{1}{64.5} \times \frac{10^6}{15{,}000} \times 3.8 \times \frac{1}{0.4}$$

$$= 2.67$$

Specific activity (SA):

$$SA = \frac{\Delta EA}{\Delta O.D._{460}}$$

Where:

ΔEA = difference between enzyme activity in each sample and the initial sample

$\Delta O.D._{460}$ = corresponding difference in turbidity between each sample and the initial sample

$$SA_8 = \frac{2.67-2.01}{0.220-0.210} = 66.0$$