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Quantitative Assessment of the Effect of Glucose, Lactose and Sucrose on the Production of β-Galactosidase

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Previous experiments measuring β-galactosidase (β-gal) activity using a colourimetric enzyme activity assay have shown that the addition of lactose to cultures induced with isopropylthiogalactoside caused an apparent decrease in induction of the enzyme. This effect stands out as counter-intuitive. We demonstrate that the production of β-gal enzyme protein by *E. coli* B23 grown in the presence of lactose is in fact higher than that of a glycerol control. This finding was determined quantitatively by measuring the amount of β-gal enzyme protein directly. *E. coli* B23 was grown in a variety of media, each containing a different carbohydrate, and culture samples were taken over a time course of 60 minutes. Proteins were extracted by bead bashing, and a Bradford assay was used to determine the concentration of protein in each sample. Amounts of β-gal were assessed by a densitometry assay following protein separation and detection by SDS-PAGE and silver staining. Despite low β-gal enzyme activity readings compared to the glycerol control, possibly due to the interference of lactose in the colourimetric assay, the densitometry readings show that this protein was present in significantly larger amounts in the lactose sample compared to the glycerol control. Growth curves for *E. coli* grown in media containing different carbohydrates were approximately the same, which ensures that differences in enzyme expression at equivalent times were not due to different amounts of bacteria in the cultures. The following experiments show that measuring enzyme activity of β-gal is an inaccurate way of determining β-gal induction when cells are grown in medium containing lactose. Instead, the amount of β-gal protein produced should be measured directly using SDS-PAGE and a suitable protein detection method.

In previous experiments, glucose, lactose, and sucrose were added to minimal media to determine how extra nutrients in the environment affect the pattern of cell growth and β-galactosidase induction. It was shown that the addition of both glucose and sucrose caused a slower induction of the enzyme than a glycerol control and interestingly, that the addition of lactose caused an even more pronounced decrease in apparent induction that remained suppressed for the duration of the experiment. This observation stands out as surprising and counter-intuitive.

It is our hypothesis that the apparent decrease in induction in the presence of lactose may be due to certain interactions taking place during the detection assay and therefore an enzyme activity assay may not be an adequate way of obtaining a clear picture of the effects of additional nutrient. Therefore, measuring the amount of enzyme protein produced, rather than enzyme activity, will provide a clear picture on the actual abundance of enzyme under the above conditions. In the following study, the amount of β-gal protein was measured directly using SDS-PAGE and silver staining techniques, with the expectation that the sample grown with lactose would have similar, if not higher, levels of β-galactosidase than in the other experimental conditions and the control.

MATERIALS AND METHODS

Bacterial Cultures: The experiment was carried out using *E. coli* B23 grown in M9 medium containing 0.2% glycerol (w/v) to a concentration of 0.186 OD_{460}.

Growth Sampling: IPTG was added to the inoculated medium to a final concentration of 0.10 mM. Samples of 3ml were taken just before IPTG was added, just after, and at 3, 6, 9 and 12 minutes. The culture was then split into three other flasks, each containing either 0.1% lactose (w/v), 0.1% glucose (w/v), or 0.1% sucrose (w/v). Samples were taken from all 4 flasks (the three nutrient flasks and the control flask) at 16, 20, 26, 32, 38, 44, 50, and 60 minutes. Turbidity readings were taken at 460 nm, and samples were toluenized and kept for use in the following assay.

β-Gal Enzyme Activity Assay: This assay was carried out as described in (1).

Bead Bashing Cell-extraction: Carried out as described in (2).

Bradford Assay: Three different dilutions of the cell-extract samples were prepared using distilled H_2O: 1, ½, ¼. BioRad® Bradford reagent was diluted 1-in-5 with distilled H_2O. 10 µl of sample was added to 100 µl of diluted Bradford reagent in each well of a 96-well ELISA plate. The standard was prepared by doubling dilutions from 100 mg/ml NEB® BSA with distilled H_2O starting at 1 mg/ml. The samples were incubated at room temperature for 5-to-10 min and read at 595 nm using SOFTmax® software and a microplate-reader. The standard curve was drawn with the 1 mg/ml and 0.5 mg/ml standards eliminated due to obscurity of the curve from the intense blue coloration.

SDS-PAGE: Samples were standardised to 1 mg of protein per lane and adjusted to 20 µl using dH_2O and 4x loading buffer. The samples were loaded onto an 8% SDS-PAGE gel and run at 200V for 41.5 min (Fig. 3). Using the molecular weight ladder, the band corresponding to β-gal (116 kDa) was determined.

Silver Staining: This was carried out with combined information from Dr. Ramey (personal communication) and from the BioRad® Silver Stain Plus instruction booklet. Gels were kept in the fixative reagent overnight. Gels were then rinsed two times in distilled H_2O with gentle agitation for 10 minutes each. Gels were then stained in developer solution for approximately 20 min (until sufficient staining was visible) with gentle agitation. The staining was stopped by rinsing gels in 5% acetic acid (v/v) for 15 min with gentle agitation. Finally the gels were rinsed in distilled H2O and dried.

Densitometry: The SDS-PAGE silver stained gel was placed under the GelDoc digital camera. The Alpha Imager® software was used to record the density of the β-gal band. The ß-gal band was identified by the molecular weight standards (Fig. 4). Density of the bands were standardized to the β-gal band of the "pre" sample (x=1); all density readings were relative to this β-gal band.

RESULTS

In Figure 1, it can be seen that growth is similar in all conditions tested. This shows that the tested carbohydrates were not inhibitory to growth, and any differences observed in enzyme induction are not a result of differences in cell number in the different experimental conditions.

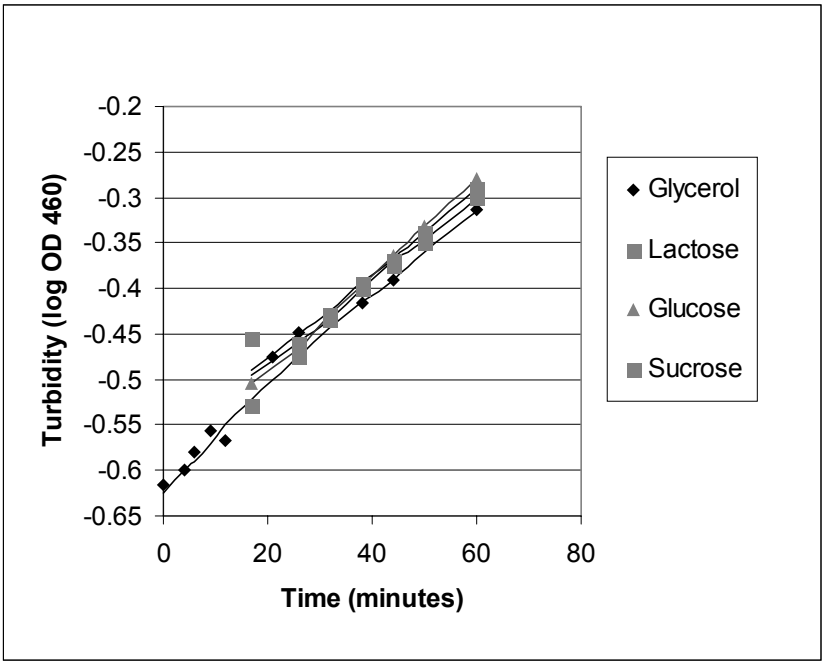

Fig. 1: Culture growth in different media containing different carbohydrates.

Induction of β-gal was measured using a colourimetric enzyme activity assay. These results are shown in Figure 2. As was demonstrated in previous experiments, the addition of glucose, sucrose, or lactose to *E. coli* induced by IPTG resulted in inhibition of β-gal enzyme activity. The most drastic inhibition of enzyme activity was seen in the lactose sample.

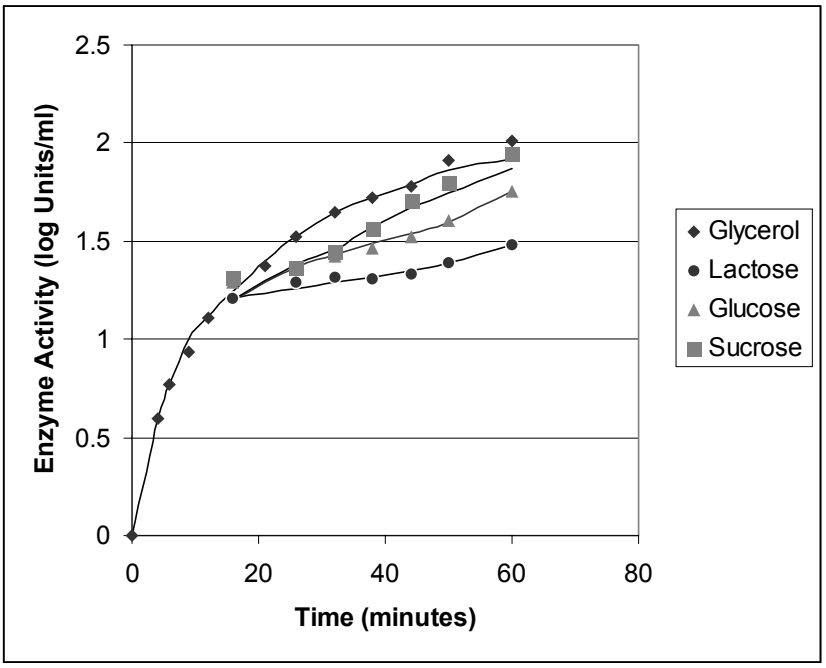

Fig. 2: Comparison of enzyme induction in different carbohydrate by enzyme activity colourimetric assay.

At the same time the samples for the colourimetric enzyme activity assay were taken, samples were also taken in which the amount of β-gal protein produced was measured directly using SDS-PAGE and silver staining techniques. The silver stained SDS-PAGE gel is shown in Figure 3. The β-gal band (116 kDa) was identified using the corresponding band in the molecular weight ladder. To ensure that the bands in the molecular weight ladder were identified correctly the log of the molecular weight (kDa) was plotted against the relative mobility (cm). As this resulted in a straight line (Fig. 4), it suggests that the bands were identified correctly. True identity could be confirmed by using a Western blot (2).

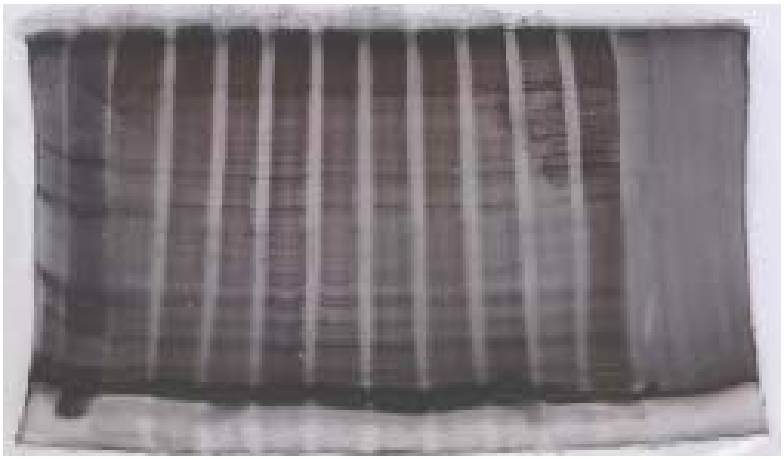

Fig. 3: Silver stained SDS-PAGE gel. Lane 1 = ladder; Lane 2 = Pre (before IPTG induction); Lane 3 = Post (after IPTG induction); Lane 4 = Gly16 (glycerol medium at 16 min); Lane 5 = Gly60 (glycerol medium at 60 min); Lane 6 = Suc16 (sucrose medium at 16 min); Lane 7 = Suc60 (sucrose medium at 60 min); Lane 8 = Glu16 (glucose medium at 16 min); Lane 9 = Glu60 (glucose medium at 60 min); Lane 10 = Lac16 (lactose medium at 16 min); Lane 11 = Lac60 (lactose medium at 60 min).

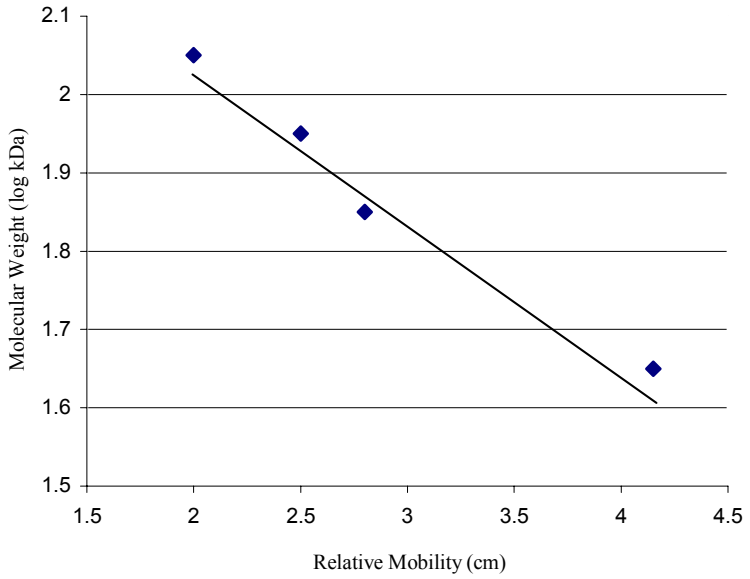

Fig. 4: Molecular Weight Standards used to determine ß-gal band.

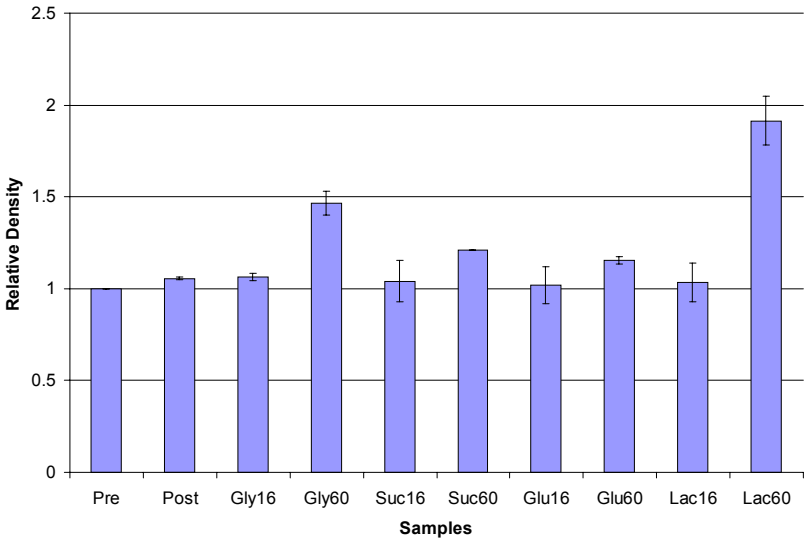

Fig. 5: Densitometry assay of β-gal production by *E. coli*. Density units are relative to the β-gal band in the "pre" sample.

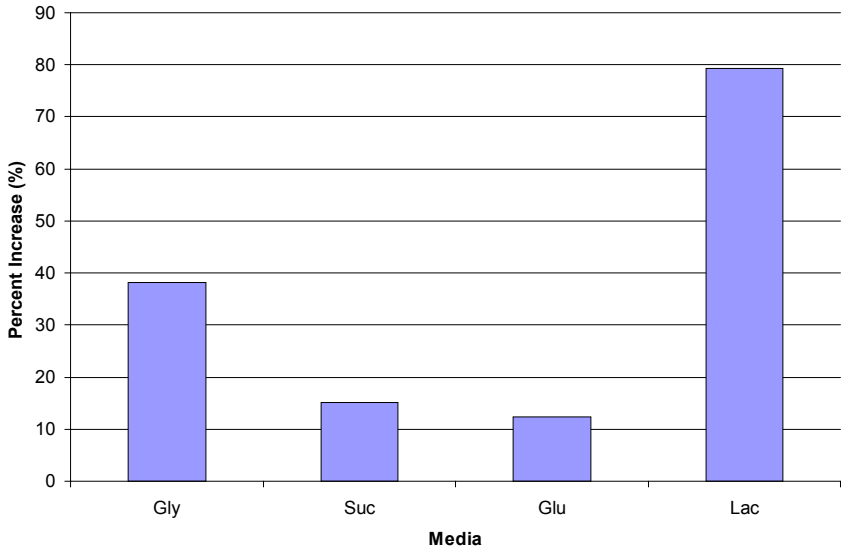

Fig. 6: Percent increase in β-gal production over 16-60 minutes by *E. coli* in media containing various sugars.

After the bands on the gel were visualized by silver staining, the bands corresponding to β-gal were quantified and their density was recorded. The density of the "pre" sample was given a value of 1, and the density of all other samples is displayed relative to that (Fig. 6). The samples from 16 minutes for each of the different growth conditions had comparable amounts of β-gal. However, the samples taken at the end of the time course at 60 minutes show different amounts of β-gal present depending on the carbohydrate added. This is seen more clearly in Figure 6 where the percentage increase in β-gal production over the 44 minute period from 16 minutes to 60 minutes is graphed. The control condition (Gly) shows an increase in production of β-gal of 38%. The sucrose and glucose samples show a decrease in induction compared to the control with only 15% and 12% increases respectively. On the other hand, the lactose sample shows an increase in induction compared to the glycerol control with a 79% increase in β-gal over the 44 minutes of growth.

DISCUSSION

Presence of different carbohydrates did not affect growth curve

To ensure that the addition of the different carbohydrates to the medium did not affect the growth rate of the cultures and thereby skew the results for the enzyme assay and SDS-PAGE gel, samples of culture were taken to monitor bacterial growth. All the cultures had similar growth curves, as shown by measuring culture optical density (Fig. 1). Thus, we can be confident that differences in enzyme expression at equivalent times were not due to different amounts of bacterial cells in the cultures.

β-gal protein levels high despite low enzyme activity in lactose medium

In the β-gal enzyme activity assay it was shown that there was an apparent decrease in induction of β-gal enzyme activity for *E. coli* grown in lactose medium compared to *E. coli* grown in glycerol medium (Fig. 2). By using silver staining and densitometry, we have shown that production of β-gal by *E. coli* grown in lactose medium (Lac) is clearly higher (79.3%) than in glycerol medium (Gly – 38.1%) over 44 minutes (Fig. 6). This demonstrates that despite a low enzyme activity reading, the β-gal protein is present at a high level in lactose medium. Therefore, the presence of lactose in the medium must be interfering with the detection of β-gal enzyme activity. For this reason, direct measurement of enzyme protein produced, rather than a determination of enzyme activity is a more accurate way of determining β-gal induction for *E. coli* grown in lactose medium. The inaccurate measurement of β-gal induction by *E. coli* grown in the presence of lactose is important to remember when using LacZ fusions as a reporter system. In this case, it would be more effective to grow cultures in medium that does not contain lactose and use an enzyme activity assay to measure the presence of β-gal, or to measure the amount of β-gal enzyme directly using SDS-PAGE and silver staining (or another appropriate detection method) if cells must be grown in lactose containing medium.

β-gal enzyme activity and production inhibited in both sucrose and glucose medium

Both the β-gal enzyme activity assay and densitometry show that the production of β-gal is repressed in medium containing either sucrose or glucose (Figs. 2 and 5). In the enzyme activity assay, next to lactose, glucose caused the most severe inhibition of β-gal activity. In medium containing sucrose, induction of β-gal as measured by the enzyme activity assay was also slightly lower than the glycerol control. A similar pattern is observed for the level of β-gal enzyme present as measured by SDS-PAGE and densitometry. In Figure 6, it can be seen that the addition of glucose resulted in the lowest percent increase over the 44 minute period (12%). The addition of sucrose also resulted in a low percent increase (15%), although it was slightly higher than that for glucose. This inhibition of β-gal induction may be a result of catabolite repression, though other mechanisms might be involved.

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Appendixes

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