**Lactose as natural alternative to Isopropyl-ß-D-1-thiogalactopyranoside, in inducing protein expression in Escherichia coli strains**

Alice Graham

## Abstract

*Escherichia coli* is the preferred organism for therapeutic protein expression, due to its quick growth and the presence of the lac operon system. To increase efficiency, cell stress must be minimised as it has a negative effect on protein expression. The lac operon is often induced by Isopropyl-ß-D-1-thiogalactopyranoside (IPTG), however in high quantities IPTG can lead to added cell stress. By using the natural inducer of the lac operon, lactose, cell stress is minimised as lactose acts also as a nutrient source. We experimented whether the efficiency of lactose is strain and concentration dependant, by setting up multiple plates of induced cells and then assessing green fluorescent protein levels and OD600 with a plate reader. We showed that lactose can induce protein expression, levels of which is concentration and strain dependent. Therefore, lactose could be used to induce multi-plasmid

## Introduction

The chromosomal lac operon of Escherichia coli is a classic example of gene regulation (Jacon and Monod, 1961), E. coli cells can control lactose metabolism by altering transcription of the lac operon. In its inactive state the lac repressor is bound to the section of DNA within the E. coli called the lac operator. In the presence of lactose molecules, in the form of allolactose, the repressor protein dissociates from the operator allowing RNA polymerase to bind, and transcription of the genes to occur.

IPTG is a chemical inducer of this process used in industry and research. During protein production, the cell undergoes physiological stress which is exacerbated by IPTG as it increases the toxic effect of the substrate upon the cell, resulting in damage to the E. coli cells (Dvorak et al., 2015). Lactose, when transformed to allolactose by beta-galactosidase, can induce the system and is a non-toxic nutrient source.

Our aim for our experiment was to both work out whether there was a significant difference in the amount of protein produced by E.coli strains, when induced with IPTG and with it's natural alternative lactose, and if there was an optimum concentration of lactose for protein production. We measured growth in OD600- cell concentration and protein production by Green Fluorescent Protein production.

## Hypothesis

Lactose will induce protein production and cell growth in E.colicells more than Isopropyl ß D 1 thiogalactopyranoside

**Materials**

Strains were grown on LB agar (Miller, L3027) plates, and grown in LB broth (Sigma-Aldrich, L3522), with antibiotic selection using 50μg/mL kanamycin (Sigma-Aldrich, K1637). Induction was performed using frozen stocks of 1mol isopropyl- β – thiogalactopyranoside (Sigma- Aldrich, 16758) and 100mM solution of lactose made by dissolving 0.68g ɑ-lactose- monohydrate (Sigma-Aldrich, L3625) in 15ml of heated milliQ water, and then filter sterilising it with a 20μm syringe filter. All *Escherichia coli* strains ( Table 1) were obtained from existing - 80°C frozen stocks within the Biocompute Laboratory at the University of Bristol

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| --- | --- | --- |
|  ***Escherichia coli* Strain** | **Origin** | **Antibiotics needed** |
| **K-12** | Biocomputelaboratory, University of Bristol | None |
| **DH10B** | Biocompute laboratory, University of Bristol | Kanamycin |
| **BL21-DE3** | Biocompute laboratory, University of Bristol | None |
| **MG1655-GFP-Burden** | Biocompute laboratory, University of Bristol | Kanamycin |
| **pTHS-VC** | Veronica Greco, Biocompute laboratory, University of Bristol | Kanamycin |

## Method

###  **Cell culture**

* Used a pipette tip to **streak out the E. colistrains** on LB Agar plates, with the correct antibiotic added in a working concentration (1000ul/mg).
* Placed the plates in an **incubator** for a maximum of 12 hours overnight at 37°C.
* Once the colonies had grown, we scraped individual colonies off the agar with pipette tips and placed them in a **96 well culture plate** (ThermoFisher Scientific, 248852), along with the appropriate **antibiotics** in working concentration and **200μl of LB media**.
* Sealed the plate with a breathable membrane and placed it in an incubator shaker (Stuart, S1505) for 12 hours at 37°C.

### **Adding reagents to three plates**

* Took **15μl of each culture and diluted it in 185 μLLB media** with the addition of kanamycin if required, in a 96-well imaging plate.
* We added strain K-12 to rows A and B, DH10β to rows C and D, BL21-DE3 to E, F and pTHS-VC to G, H. To columns 4-6 we added 0.2μlof 1Mol IPTG, to columns 7-9 we added 0.6 μlof IPTG and 10-12 we added 1 μlto the 200 μltotal media and cell volume. **For rows B, D, F and H we added 0.2 μllactose solution as well as IPTG.**
* We used a **BioTekSynergy Neo2 plate reader** to take readings at 37°C for 12 hours overnight every ten minutes, for **Green Fluorescent Protein Level** and **OD600**.
* We set the light wavelengths at 479,520 for the readings.
* Plate 2 and 3 cells were induced by only the 100mM lactose solution. We wanted to test the green fluorescent protein (GFP) levels and optical density of a sample measured at a wavelength of 600nm (OD600) of strains; BL21-DE3, MG1655-GFP-Burden and pTHS-VC at lactose concentrations(mM); 20, 40, 45, 50, 55, 60, 65, 70, 75, 80.
* Three repeats for each strain and concentration as well as **controls for each strain** with 0mM lactose added.

### **Data analysis**

* Data from the readings was standardized and analyzed using **Rstudio** (RstudioTeam, 2010)
* **GFP expression rate calculated** using the equation GFP expression ratet2 = ((total GFPt3) –(total GFPt1)/(t3 –t1))/ODt2 where t1 = 30 min, t2 = 60 min and t3 = 90 min after induction (Ceroniet al, 2015).

## Results

## Fig. 1a

## Fig 1b

No significant difference that Green Fluorescent Protein production differed per strain when 1mM lactose was added in 1b (Chi-squared = 6.5515 p-value = 0.08765, df= 3). Escherichia colistrains BL21-DE3, DH10B-GFP, pTHS-VC and K-12.  Readings taken with BioTekSynergy Neo2 plate reader at 37°C for 12 hours overnight, wavelengths 479,520. Error bars on figure 1a and 1b calculated with 95% confidence intervals.