The Effect of Varying Promoter Strengths on the Cost and Benefit of the Lac Operon

Katarina Zahedi
Edgar, M., Duckweed Tigers, 01/26/18

Abstract: The goal of this experiment was to observe how varying promoter strengths of the lac operon in strains of *Escherichia coli* affects the cost and benefit outcomes, quantified as the relative growth rate difference of IPTG-induced to uninduced bacteria. The amounts of lactose given to each strain were varied to produce lactose response curves in order to observe the cost-benefit effects after 24 hour growth in a rolling incubator. The absorbance values were measured using a spectrophotometer (OD600), and the cost-benefit outcomes were determined by calculating the growth rate difference with a positive value indicating benefit and negative indicating cost. Based on the collected data, weaker promoters tend to incur less cost and more benefit than stronger promoters. In the weak promoter strain, the greatest benefit with least cost was present at 400 μM of lactose, reaching 0.0322 ± 0.03 for growth rate difference, and the greatest cost with least benefit was present at 200 μM of lactose in the strong promoter strain, reaching -0.0905 ± 0.03. In addition, observed trend lines show the strains with weaker promoters having overall less cost. Because stronger promoters have a higher level of gene expression, they create significant metabolic load on the lac operon, incurring cost through unnecessary expression and thus exhibiting a need for weaker promoters as seen in general lactose response curves of strong, medium, and weak promoter strains from lab results.

Introduction: The lac operon is a group of genes that are transcribed together as mRNA, and this mRNA is later translated into proteins, such as β-galactosidase, permease, and transacetylase, that allow the bacteria to use lactose as an energy source.[1] The operons control gene expression, which is the production of a protein from a gene.[2] LacZ, a gene that plays an important role in this lab, is transcribed into the part of mRNA that encodes β-galactosidase, which is an enzyme that breaks lactose down into glucose and galactose.[3] LacY, another notable gene in this experiment, is transcribed into the part of mRNA that encodes permease, which is a protein that transports lactose into the bacterium cell.[4] There are also regulatory sequences of DNA, such as the promoter, that control transcription and help determine whether the operon is turned 'on' or 'off.'[5] The promoter is the binding site for RNA polymerase, which is the enzyme that performs transcription; the strength of the promoter, which plays an influential role in probability of transcription, can be defined by its affinity for RNA polymerase.[6] Because of these regulatory sequences, the combination of lactose and no glucose given to the bacterium allows for the lac operon to be expressed at high levels and perform strong transcription.[7] However, the cell must maintain a balance between the cost (relative decrease in cell growth rate) and benefit (relative increase in cell growth rate) of the protein expression of the lac operon.[8] [9]

Thus, the goal for this lab was to observe how varying promoter strengths of the lac operon affects the cost and benefit outcomes, quantified as the relative growth rate difference of IPTG-induced to uninduced bacteria, as a means to answer the experimental question: In engineered strains with strong, medium, or weak promoter of the lac operon, the promoter of which strain type produces the most benefit with least cost at varying lactose? It was hypothesized that the engineered strain with the strong promoter will produce the most benefit with the least cost, and that the strains with the medium and weak promoter will both have more benefit than cost but not as much as that of the strong promoter.

---

1 Edgar, M. (2017). *SCAB-2: Advanced Biology,* Lac Operon (Bacteria) Presentation notes [PowerPoint slides]. Milton Academy, Milton, Massachusetts 02186.

2 Ibid.

3 Kalisky, T., Dekel, E., and Alon, U. 2007. Cost-benefit theory and optimal design of gene regulation functions. Phys Biol 4:229-245.

4 Ibid.

5 Penumetcha, P., et al. 2010. Improving the lac system for synthetic biology. Bios 81(1):7-15.

6 Brewster, R., Jones, D., and Phillips, R. 2012. Tuning promoter strength through RNA polymerase binding site design in Escherichia coli. PLoS Comput Biol 8(12): e1002811.

7 Tanase-Nicola, S. and ten Wolde, P. 2008. Regulatory control and the costs and benefits of biochemical noise. PLoS Comput Biol 4(8): e1000125.

8 Dekel, E. and Alon, U. 2005. Optimality and evolutionary tuning of the expression level of a protein. Nature 436:588.

9 Eames, M. and Kortemme, T. 2012. Cost-benefit tradeoffs in engineered lac operons. Science 336:911-915.

Methods and Materials: In order to evaluate the hypothesis that the strong promoter strain would produce the most benefit with the least cost, the amount of lactose given to the bacteria was varied in order to produce lactose response curves. The promoter strength was varied by using a bacterial strain (from previous ONPG lab with Mr. Edgar) of either a strong, medium, or weak promoter, with all strains having a constant ribosome binding site strength (strong). The ribosome binding site strength was controlled so that the difference in results are caused by differing promoter strengths. The external lactose concentrations (0, 200, 400, 600, 800, 1000 μM) were made from a lactose stock concentration of 15000 μM. This stock concentration was made using 0.1 g of lactose powder and 20 mL of distilled water. The concentrations for the varying external lactose and the IPTG solution (500 μM) were chosen based on Eames and Kortemme (2012). The concentration of IPTG was controlled so that all treatments would have the same level of expression after undergoing similar strengths of induction by IPTG. The dilution (1:100) for the bacteria stock solutions (made from bacteria grown overnight in shaking water bath and Luria Broth) of strong, medium, and weak promoters was decided based on Mr. Edgar's suggestions. The dilution of bacteria was controlled so that one strain type would not have more bacteria than another in order to ensure that differences in bacteria growth were caused by varying promoter strengths. All solutions of bacteria, lactose, and IPTG concentrations were made in preliminary testing and were also used in final testing. Through preliminary testing, the best method for carrying out this experiment was determined. Because there were no issues with bacteria growth or producing clear results through the chosen method used in preliminary testing, the same method was used in final testing. As shown in Figure 1, the lab was set up using 96-well plates, with each plate containing two trials. There were six trials in total, with the three treatment groups being the strong, medium, or weak promoter bacteria each given varying lactose concentrations (previously stated). There was also two sets per trial: a set given IPTG and one without IPTG as a means to quantify the relative growth rate difference between the two. Each well contained 200 μL of strong, medium, or weak promoter bacteria solution (treatment), 50 μL of either IPTG or no IPTG (given distilled water instead), and 50 μL of varying lactose concentrations. The amount of each solution type in the well was controlled as a means to isolate the effect of promoter strength on cost-benefit outcomes. The negative control of no bacteria (blank) was made by adding distilled water instead of bacteria solution to the well. This control was used to ensure that none of the media was contaminated and that changes in growth rate difference were caused by differing promoter strength because without any bacteria, the blank well were expected to have zero growth. After filling the wells with their designated types of bacteria, IPTG/no IPTG, and lactose concentration, the well plate was then placed in a rolling incubator at 37℃ to grow for 24 hours. The overnight time and temperature conditions were controlled so that differences in results would not be caused by more time or heat given to bacteria for growth as a means to focus solely on the effects of promoter strength on cost-benefit outcomes. After overnight growth, O.D. measurements (600 nm) of the bacteria using a spectrophotometer were taken, and the cost-benefit outcomes were calculated by finding the growth rate difference of IPTG-induced relative to uninduced bacteria strain set. When the growth rate difference is greater than zero, this implies benefit; when the growth rate difference is less than zero, this implies cost. Since the data was quantitative, multiple t-tests were chosen to evaluate significance, comparing all lactose response curves to each other as well as individual points of the lactose response curves to give a more in depth insight.

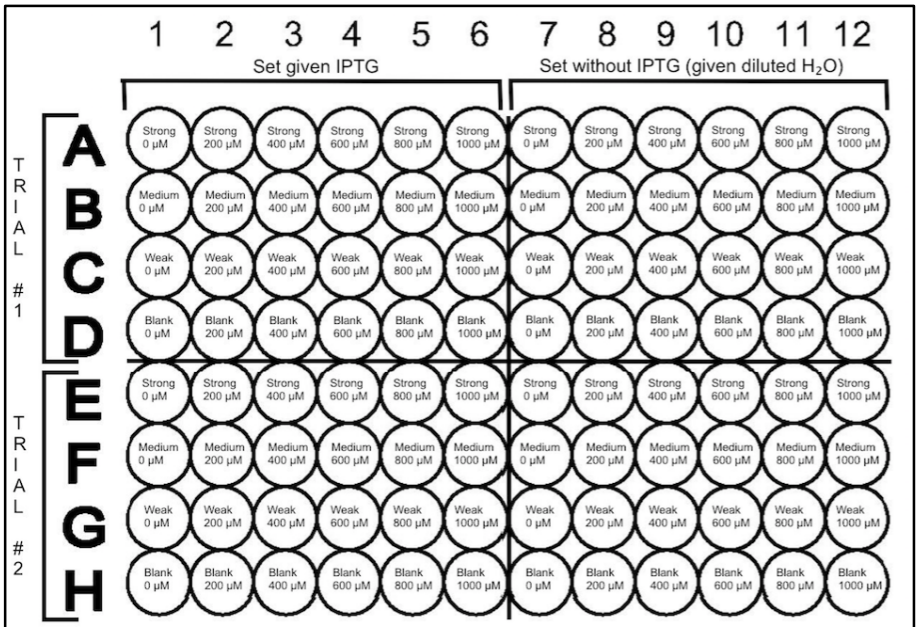


Figure 1. This diagram shows the setup of the experiment. As seen above, each 96-well plate contains two trials, with each trial having a set of liquid cultures with IPTG and a set without IPTG (given diluted water instead). Strong, medium, and weak labels represent the promoter type of the bacteria strain. Blank label represents negative control (no bacteria). 0, 200, 400, 600, 800, and 1000 μM labels display the varying lactose concentrations.

Results: The engineered strain with the weak promoter was less costly than the other two strains, but also had little benefit overall; both the strong and medium promoter strains had no benefit at all, only incurring cost from protein expression of the lac operon. The results from Figure 2 reveal the relationship between the promoter strength and the cost-benefit outcomes. Each growth rate difference value at the set lactose concentrations on the graph represents the average of six trials of growth rate difference values measured/calculated. The negative control of no bacteria (blank) had zero growth and thus had neither cost nor benefit. The strain with the weak promoter followed a trendline that had less cost than the other strains; however, there was also little benefit overall, with the greatest relative growth rate difference being 0.0322 ± 0.03 when given 400 µM of lactose. The weak promoter strain when given 400 µM of lactose was the only instance where there was much more benefit than cost in protein expression of the lac operon. However, at 1000 µM of lactose in the weak promoter strain, the growth rate difference did go above zero, but there was only a very minimal amount of benefit (0.0042 ± 0.04).

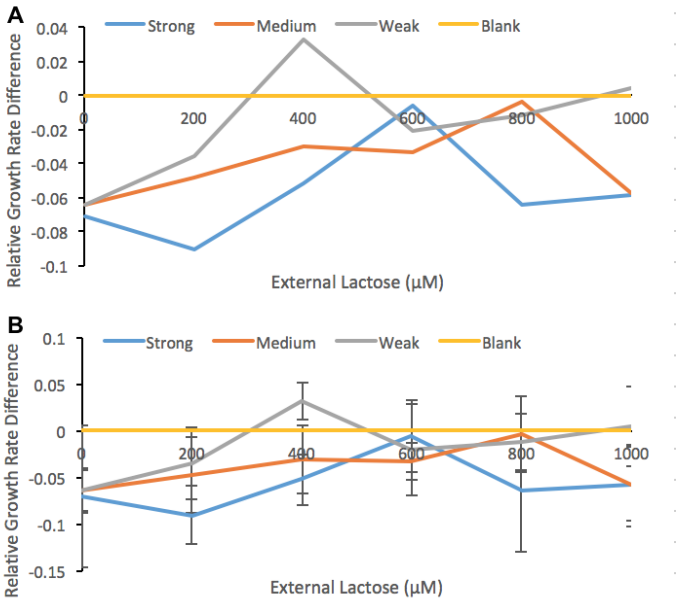


Figure 2. Cost/benefit tradeoffs in Escherichia coli strains of varying lac operon promoter strengths. Cost/benefit measured by the mean (n=6) relative growth rate difference (±AAD) of IPTG induced bacterium relative to uninduced at varying lactose treatments to produce lactose response curves. Treatments: Negative control (blank; no bacteria), strong promoter, medium promoter, and weak promoter of the lac operon. (A) No uncertainty bars present and smaller axes range for more in-depth graph. (B) Greater axes range to include uncertainty bars.

While the greatest benefit was found in the weak promoter strain, the greatest cost was observed in the strong promoter strain when given 200 µM of lactose, with the growth rate difference being -0.0905 ± 0.03. The overall trendline of the strong promoter strain incurs more cost than the overall trendline of the medium promoter strain, but at 600 µM of lactose, the strong promoter strain (-0.0062 ± 0.04) has less cost than the medium promoter strain (-0.0330 ± 0.02). Overall, only the strong promoter is significantly different than the medium ($p<0.0006$) and weak ($p<0.0013$) promoter strains, whereas there was no significant difference between the medium and weak promoter strains. Looking at individual data points, the only significant difference was between the strong and weak promoter strain at 400 µM of lactose ($p<0.0009$) and between the medium and weak promoter strain also at 400 µM of lactose ($p<0.0329$). For all engineered strains, it appears that the relative growth rate difference reaches a greatest value (with the least amount of cost and possibly some benefit) at a certain concentration of lactose and then decreases as the lactose concentration increases. The overlapping uncertainties in the trendlines showed that there is not a major difference between the growth of each strain type (Fig 2B).

Discussion: The experiment's outcome does not support the hypothesis that the engineered strain with the strong promoter will produce the most benefit with the least cost, and that the strains with the medium and weak promoter will both have more benefit than cost but not as much as that of the strong promoter. The actual outcome had little instances of greater benefit than cost across all strain types, and the weak promoter strain actually had less cost than the other two strains and only had some benefit while the strong and medium promoter strains had no benefit at all and only cost from protein expression of the lac operon. The results of this lab occurred rather than hypothesized results possibly because the effects of other genes in the lac operon were not taken into account, such as lacY's production of permease and permease activity. Permease activity, based on articles discussed in class, incurs more cost than lacZ's production of β-gal and β-gal activity because of permease leakiness in transporting IPTG and lactose into cell,[10] thus disproving the hypothesis and its rationale that a stronger promoter leads more benefit because there will be higher levels of β-gal activity and thus more cell growth. While it may be true that higher levels of β-gal activity are associated with increased cell growth, it is not true that this is the major source behind the cost of protein expression. Because strong promoters tend to express genes at the highest levels, there is a significant metabolic load on the lac operon and the bacterium, thus exhibiting a need for weaker promoters since bacteria with unnecessary gene expression (due to stronger promoters) will most likely be selected against in a

[10] Eames, M. and Kortemme, T. 2012. Cost-benefit tradeoffs in engineered lac operons. Science 336:911-915.

natural environment. Unneeded expression is costly, thus decreasing organismal fitness because unnecessary transcription of one set of genes can reduce the level of transcription of other genes.[11] The same goes for translation, in that nonessential translation of one protein can reduce translation rates of another protein.[12] Therefore, a weaker promoter should incur less cost and more benefit than a stronger promoter and vice versa, as shown in the results of this experiment. In accordance with this rationale, Figure 2 showcases that the overall trendline of the weak promoter strain has less cost than that of the strong and medium promoter strains, while the medium promoter strain has less cost than the strong promoter strain. At 400 μM of lactose, the weak promoter strain shows the greatest benefit (0.0322 ± 0.03) out of all data points, whereas the medium promoter strain (-0.0305 ± 0.04), while not having any benefit, still exhibits less cost than the strong promoter strain (-0.0518 ± 0.03) (Fig 2), thus justifying the reasoning that stronger promoters tend to incur higher cost than weaker promoters due to unnecessary gene expression. Because the data points at 400 μM of lactose were significant ($p<0.0009$ in comparing strong promoter and weak promoter; $p<0.0329$ in comparing medium promoter and weak promoter) and the trendlines comparing strong promoter and weak promoter were also significant ($p<0.0441$), the results of the lab support the idea that weaker promoters will incur less cost and more benefit than stronger promoters, thus disproving the original hypothesis.

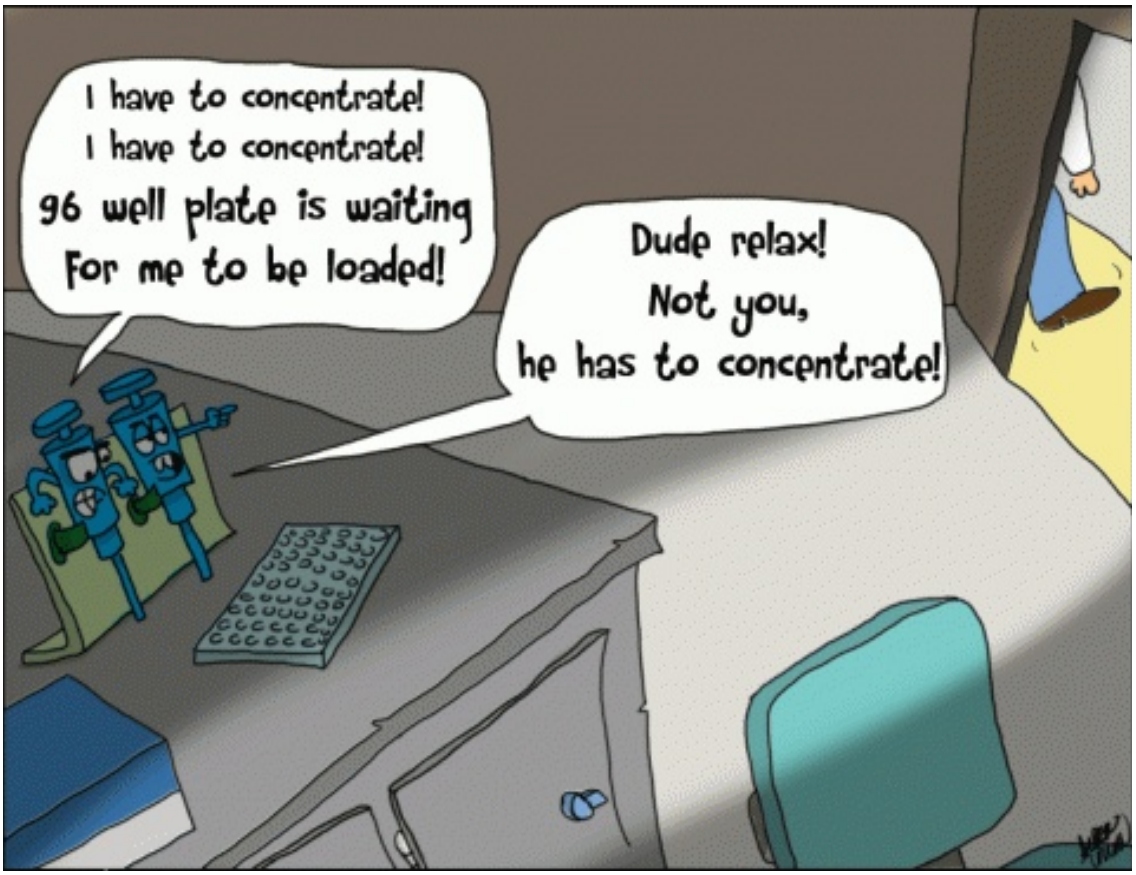
Major sources of uncertainty in this lab resulted from contamination, difference in bacteria amount in stock solution (made before 1:100 dilution), and the amount of each solution pipetted into a well. The cultures in each well might have been contaminated due to the micropipette tip touching the edges of one well filled with one type of bacteria and then using the same pipette tip in a well with another type of bacteria when adding IPTG solution, which could affect data by accidentally mixing two types of bacteria strains together. To prevent contamination, a new pipette tip could be used if the previous one accidentally touches the side of the well, or the other hand could be used to steady the micropipette so that the pipette tip would not accidentally touch the edge of the well. The bacteria amount in each stock solution might have also varied between bacteria strain types because the amount of bacteria taken up by the loop was in greater amount for some strains than others, which could affect the alter the trends observed by affecting the bacterial cell density after overnight growth. To maintain roughly the same amount of bacteria inoculated on the loop, a set number of swipes across the bacterial surface could be determined because for some strains, a back and forth swipe was carried out while for other strains, only a singular swipe was done. The final major source of uncertainty was the amount of each solution (bacteria type, IPTG/no IPTG, lactose concentration) pipetted into a well. Because volume units are remarkably small (μL), even a bubble present in a pipetted solution could influence the outcomes of the lab. Thus, by simply making sure that there are no bubbles in the liquid that has been pipetted, this uncertainty could be prevented in order to produce more consistent results.

A further experiment could explore the relationship between lactose concentration and cost-benefit outcomes since the ideal promoter strength for cost-benefit effects has been determined from this experiment, assuming more benefit can be produced from the weak promoter strain. This future lab could be carried out by varying the lactose concentrations as means to determine the best lactose concentration given to bacteria that will allow for the most benefit with the least cost. However, more runs would need to be created for lactose concentration, and the ideal ribosome binding site strength of the lac operon might also need to be determined in preliminary testing before carrying out future experimentation. In order to make this future experiment viable, a method for decreasing uncertainty to produce more accurate and consistent results would need to be designed. This next step could allow for improvement in protein production efficiency of the lac operon and overall organismal fitness of the cell.

As presented by the results, the outcome of this experiment may not support the hypothesis, but significant trends were discovered: weaker promoters tend to incur less cost and more benefit than stronger promoters.

[11] Stoebel, D., Dean, A., and Dykhuizen, D. 2008. The cost of expression in Escherichia coli lac operon proteins is in the process, not in the products. Genetics 178:1653-1660.

[12] Ibid.



P.S. Sorry, Mr. Edgar, but I am never using a well plate again. It hurts my brain more than trying to understand the actual biology itself.