The Roles of GalS, GalR and GalK in the Regulation of Glucose and Galactose Metabolism in *Escherichia coli*

MONICA WANG AND BARBIE PETERS
General Microbiology Laboratory PMB/MCB C112L, University of California, Berkeley
Berkeley, California

October 30, 2023

Based on Jacques Monod's thesis, it is known that *Escherichia coli* is capable of performing multiple sugar metabolism, though prefers to consume one sugar at a time in a process called carbon catabolite repression (1). Additionally, *E.coli* prefers glucose consumption first over other C sources and only after fully consuming glucose does it begin to consume the alternative C sources. In this work, we characterized a second hierarchy in *E. coli* – one between glucose and galactose – and how *galR, galS, and galK* genes affect *E.coli's* ability to perform galactose metabolism. To determine the effects that these genes have on *E.coli's* galactose metabolism, we performed growth and Biolog assays for WT and three *E.coli strains* that have single-gene knockouts for *galR, galS, and galK*. We found that *galR and galK* are essential genes. While *galS* was found to be non-essential genes for galactose metabolism.
Not only do these results give us a better understanding of the role that *galR, galS, and galK* genes play in *E.coli,* but these results may be helpful in the advancement of metabolic engineering of E. coli strains capable of producing chemical and biofuels from mixtures of hexose and pentose sugars derived from plant biomass.

INTRODUCTION

Escherichia coli is a common and frequently used model bacteria in scientific research to gain a better understanding of genes, functions, and much more. *E. coli* is a Gram-negative, rod-shaped, facultative anaerobic bacterium that usually colonizes the gastrointestinal tract of humans and animals (7). Among the *E. coli* strains used to research, a common laboratory strain used is *E. coli* BW25113 which was created in the laboratory of Barry L. Wanner and consists of approximately 4,400 genes grouped into an estimated 1500-2000 operons (5, 7). While much is already known about *E. coli* based on the massive amounts of research papers using *E. coli* as their model organism for the experiment of interest, there is still much to learn from this bacteria and about the bacteria.

The ability of *E. coli* to adapt to a variety of environmental conditions has always been an interest to the microbiologist's world. One notable example of its adaptive characteristic is revealed when *E. coli* is grown in the presence of glucose and another sugar (6). In such a mixture of C sources, *E. coli* often consumes glucose first before consuming the other sugar source as *E. coli's* main preferred carbon source is glucose (7). This phenomenon results in a biphasic growth curve and a diauxic lag for *E. coli* where one can see two periods of exponential growth due to the stunt in growth during the diauxic lag period where *E. coli* switchover from glucose to the other sugar source metabolism (7). During this diauxic lag period, *E. coli* most likely activates gene expression of certain genes required for the generation of the necessary enzymes and proteins to metabolize the other sugar source which may take some time, resulting in the plateau between the two exponential growth. While the growth curve of *E. coli* in a

solution containing two kinds of sugars reveals information about its rate and ability to switch over to another C source, the curve reveals little about the actual mechanisms and pathways behind the curve observed.

For this paper, we will be looking deeper into this growth pattern found in *E. coli* when grown in mixtures containing glucose and galactose and focus on how certain genes involved in the *gal* regulon in *E. coli* enable the bacteria cell to consumes galactose as a secondary source of energy after glucose consumption (8). Specifically, this paper will look at the effects of *galR*, *galS*, and *galK* on *E.coli's* growth. To investigate this question, we grew and measured different growth curves of *E. coli* strains (WT, *ΔgalR, ΔgalS, ΔgalK*) from the Keio collection in media of varying glucose-galactose sugar concentrations using 96-well plate readers and Biolog assays.

According to previous research done, GalR and GalS selectively represses the downstream promoter of, P1, of the *gal* operon and slightly activates the upstream promoter, P2, in vitro (6). Additionally, it was found that GalS was 15-fold more sensitive to galactose than GalR (6). As for GalK, it is involved in the catalyzation of the first step in the Leloir pathway of galactose catabolism. From this, we initially hypothesized that *galR, galS,* and *galK* are all essential genes for galactose metabolism. With this in mind, we inferred that *E. coli ΔgalR or ΔgalS* would result in only one large exponential growth curve with no diauxic lag as enzymes for galactose would be constantly produced. While for *E. coli ΔgalK,* we inferred that the growth curve would result in one small exponential growth curve that plateaus after *E. coli* fully consumes glucose. While there is much known about the function of these genes and enzymes, it is still not fully clear how these genes and enzymes work together in relation to galactose metabolism in the Leloir pathways.

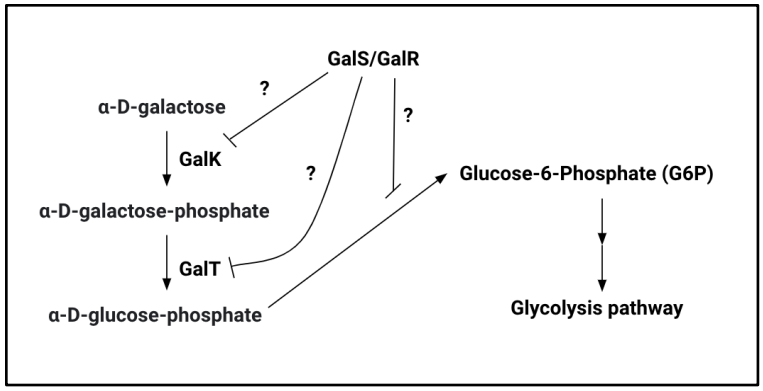


Figure 1. Regulation of galactose metabolism and relationship to glycolysis pathway. Enzymes involved in galactose metabolism include GalK (activator), GalR (repressor), and GalS (repressor).

In this work, we explored how metabolic pathways of galactose and glucose are regulated by genetic control of enzyme production. To investigate this question, we grew and measured different growth curves of *E. coli* strains (WT, *ΔgalR, ΔgalS, ΔgalK*) from the Keio collection in media of varying glucose-galactose sugar concentrations using 96-well plate readers and Biolog assays. From this, we were able to demonstrate that *galR and galK* are essential genes, while *galS* was found to be non-essential genes for galactose metabolism.

MATERIALS AND METHODS

Bacterial strains. Four *E. coli* strains were used for this paper. These four *E. coli* strains are all from the Keio collection and are isogenic derivatives of BW25113 (WT) – BW25113 (WT), JW2805 (*ΔgalR),* JW2138 (*ΔgalS),* and JW0740 (*Δgal)* (3). More information on these strains and their genotypes are given in Table 1.

TABLE 1. Bacterial strains and genotypes*

Strains	Genotype
JW2805	*ΔgalR::kan*
JW2138	*ΔgalS::kan*
JW0740	*ΔgalK::kan*
BW25113	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514* (wild type)

*All strains used are isogenic derivatives of BW25113 (wild type).

Growth culture. For the experimental growth cultures, 4ml seed culture of each *E.coli* strains (WT, *ΔgalR, ΔgalS, ΔgalK*) were used and grown overnight at 37°C in 0.2% glucose 1X MOPS minimal medium with the following concentrations and components: 1X MOPS stock solution, 1.32 mM K_2HPO, 0.2% glucose, 20mM NH_4Cl, and autoclaved nano-water (2).

From these overnight-grown seed cultures, each *E. coli* strains (WT, *ΔgalR, ΔgalS, ΔgalK)* were then inoculated into 1ml 1X MOPS minimal medium containing 0.035% glucose and 0.075% galactose with a starting *E. coli* OD of 0.05. This gave us 4 tubes each with 1ml of 0.035% glucose and 0.075% galactose 1X MOPS minimal medium and one of the *E. coli* strains (WT, *ΔgalR, ΔgalS, ΔgalK)* with OD of 0.05. Similar to the 0.2% glucose MOPS medium used to grow the overnight *E. coli* strains, the *E. coli* growth cultures were also grown in MOPS minimal medium containing 20mM NH_4Cl and 1.32mM K_2HPO_4. Each growth culture was inoculated with the *E. coli* strain with a starting O.D. of 0.05 which was calculated by using $c_1v_1 = c_2v_2$ and the stock OD (aka OD of overnight cultures) measured from Spectronic 20 spectrophotometer at 620 nm. Using these growth cultures each with one of the four *E. coli* strains, we performed the growth curve and Biolog Assay.

Measuring growth curve and rates using a 96-well plate reader. Each *E. coli* strain (WT, *ΔgalR, ΔgalS, ΔgalK)* was plated in triplicates on the 96-well plate with each well containing 150 ul of test culture of 0.035% glucose and 0.075% galactose 1X MOPS minimal medium and *E.coli* starting OD of 0.05. Growth curves and rates for the *E. coli* strains studied were measured using Thermo Scientific Multiskan FC plate readers under 37°C for 18 hours while being shaken in their growth medium. Growth was monitored and recorded every 10 minutes by checking each strain's O.D. at 620 nm. With these measurements, we were able to make a growth curve and calculate the doubling time of each strain using Excel.

Biolog Assays. Each *E. coli* strain (WT, *ΔgalR, ΔgalS, ΔgalK)* from inoculating fluid with bacteria OD of 0.01-0.05 was plated in GenIII 96-well Biolog plates that contained 74 C sources

and 23 resistance tests. Each well had 100ul of IF with *E. coli* strain. Prior to Biolog reading, the plates were maintained at 4°C before incubating at 37°C for 24hrs. Biolog well turns purple when *E. coli* utilizes that C source as glucose presence activates ETC and causes tetrazolium reduction that gives a purple product. With this experiment, we can test for our mutant phenotypes and access the mutant's ability to use C sources. After the 24hrs incubation, we read the GenIII plates and measure the OD.

RESULTS

Galactose metabolism of E. coli WT and galS, galK, and gal R mutant. Using overnight grown *E. coli* strains in MOPS media containing 0.2% glucose, we inoculated fresh 0.035% glucose and 0.075% galactose MOPS media with each *E. coli* strains (WT, *ΔgalR, ΔgalS, ΔgalK)* at starting OD 0.05 and measured their absorbance every 10 minutes for 18 hours using a plate reader. Using these measurements, we determined the growth curve and growth rate for each *E. coli* strain. The growth curve of WT and mutant *E. coli* in MOPS media of 0.035% glucose and 0.075% galactose reflect the functions of these mutant genes in aiding and utilization of galactose metabolism in *E. coli* (Fig. 2). The growth rates for each strain's exponential growth phase can be seen in Table 2.

Based on prior knowledge, we initially hypothesized that *galR, galS,* and *galK* are all essential genes for galactose metabolism. With this in mind, we inferred that *E. coli ΔgalR or ΔgalS* would result in only one large exponential growth curve with no diauxic lag as enzymes for galactose would be constantly produced. While for *E. coli ΔgalK,* we inferred that the growth curve would result in one small exponential growth curve that plateaus after *E. coli* fully consumes glucose. However, after doing the experiment, this proved to be not fully true.

Based on our experiment, *galK* and *galR* seem to be essential genes. For our growth curve for *E. coli ΔgalR,* there is no biphasic growth curve and has a final absorption value similar to the WT control which is consistent with our hypothesis and supports that *galR* is an essential gene for galactose metabolism. As for our growth curve for *ΔgalK* strain, the curve shows no diauxie lag and no biphasic growth curve which is also consistent with what we hypothesized. We infer that the one and only exponential growth phase of the *ΔgalK* strain is most likely caused by the *E. coli* strain consuming glucose for energy. Having no second exponential phase supports our hypothesis that the *galK* gene is essential for galactose metabolism as *E. coli ΔgalK* is unable to activate genes and produce enzymes necessary for galactose metabolism. Our WT control growth curve also further supports our hypothesis of *galK* being an essential gene as it has a higher final absorption value than *E. coli ΔgalK* which we believe is due to the fact that the WT has a functioning *galK* gene (Fig. 3).

While our hypotheses were supported by our data for *galK* and *galR*, *galS* was proven to be a nonessential gene. The growth curve for *E. coli ΔgalS* has a biphasic growth curve which indicates that there was diauxie lag. Because there was a lag, that mean that there was an active repressor of galactose metabolism despite *galS* being unfunctional. From this, we inferred that *galS* is unessential as there is another repressor that can be activated.

While this data supports our hypothesis that *E. coli galK* and *galR* are essential genes and disproves our hypothesis that *galS* is an essential gene, we still know little of how *galK*, *galR*, and *galS* interact together and are regulated. In further research, the interaction between these genes and regulation of expression could be deeper studied and help further advance our understanding of *E. coli* metabolism processes.

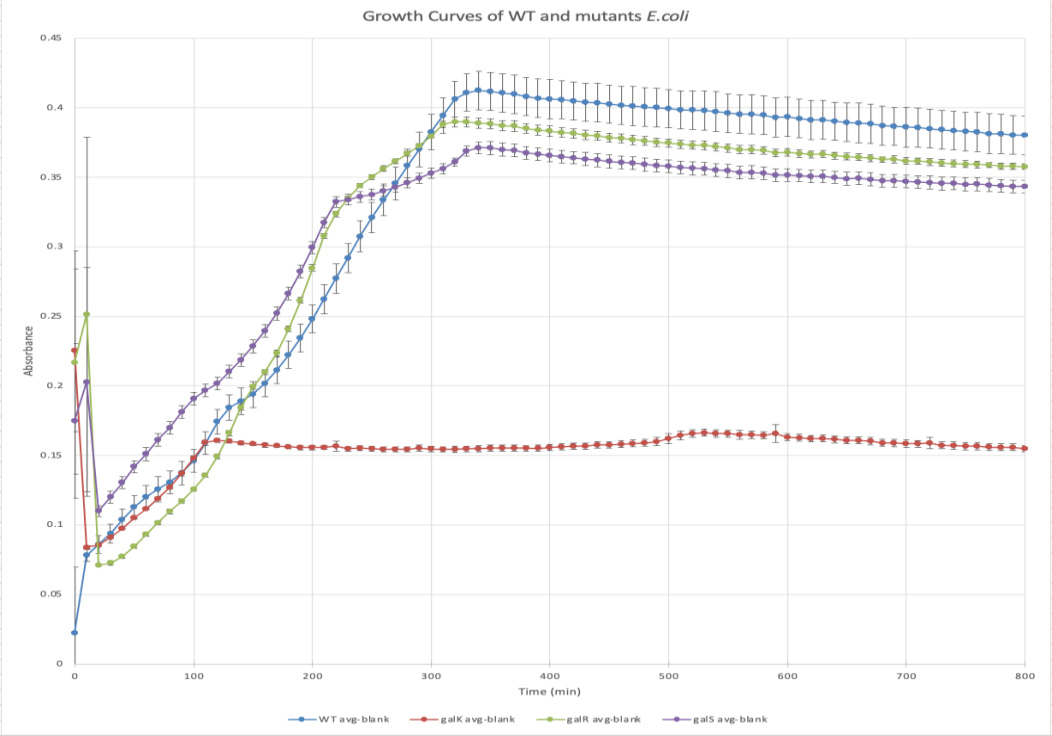
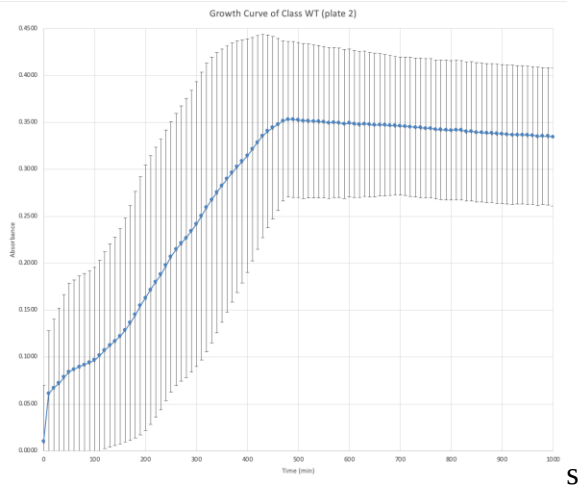


FIGURE 2. Growth curve of WT, *ΔgalR, ΔgalS, ΔgalK E. coli* strains in MOPS media of 0.035% glucose and 0.075% galactose.



s

FIGURE 3. Control growth curve of the average WT growth of the class. Using plate 2, developed a growth curve of WT in MOPS media of 0.035% glucose and 0.075% galactose.

Display steady double time growth from ~10 min to around 475 min and plateauing growth afterwards.

Strain	Growth Rate #1	Lag Time #1 (min)	Growth Rate #2	Lag Time #2 (min)
WT	133.61	10	NA	NA
ΔgalR	118.17	20	NA	NA
ΔgalS	125.40	20	753.23	0
ΔgalK	81.32	10	NA	NA

TABLE 2. Growth rates of WT and three mutant strains (*ΔgalR, ΔgalS, ΔgalK).* WT, *ΔgalKR,* and *ΔgalK* experienced no second exponential growth phase while *ΔgalS* does have a second exponential growth phase (aka biphasic growth curve).

E. coli WT and galS, galK, and galR mutant utilization of a variety of C sources. Using a Gen III Microplate, we tested our *E. coli* strains' phenotype and ability to use a variety of C sources (Fig. 4). For more details of how we performed these experiments, look in the materials and methods section.

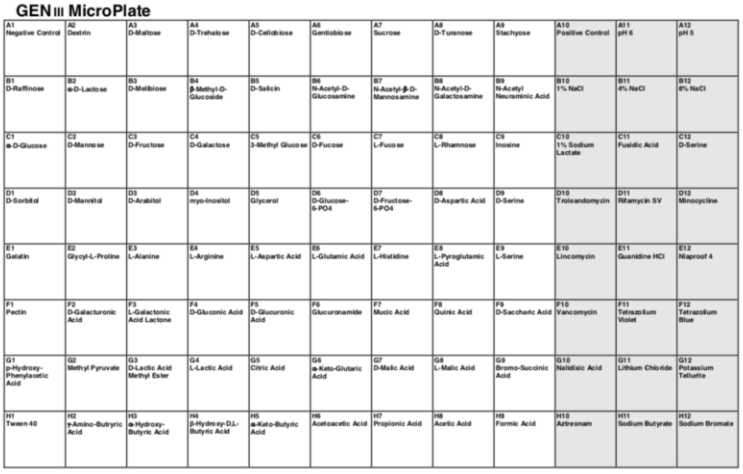


FIGURE 4. GenIII Microplate template with corresponding C sources in each well in the 96-well plate that was read by a Biolog plate reader. Using this template plate we added our *E. coli* strains, one strain per plate.

While the Biology assays look pretty similar for all the *E. coli* strains, one notable difference is with the *ΔgalR* strain. For the chemical sensitivity of tetrazolium blue, *E. coli ΔgalR* has very low absorbance value in comparison to the other strains. We infer that this is most likely caused by *galR* being an essential gene for galactose metabolism as the absorption value would have been higher when glucose is present aka galactose metabolism is active. Because *E. coli ΔgalR* has an unfunctional *galR* gene, it is unable to perform galactose metabolism resulting in the lighter color. The Biolog assay further proved that *galR* is an essential gene.

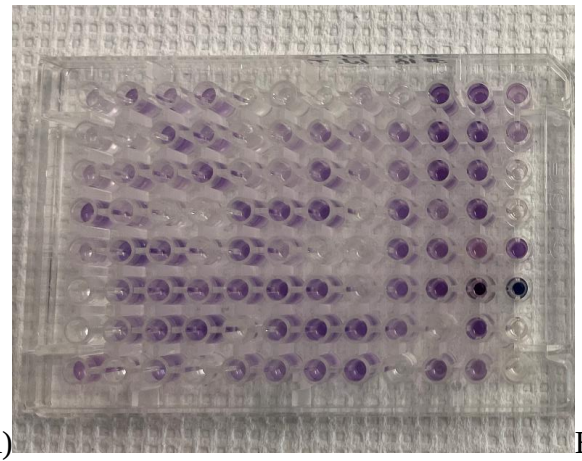
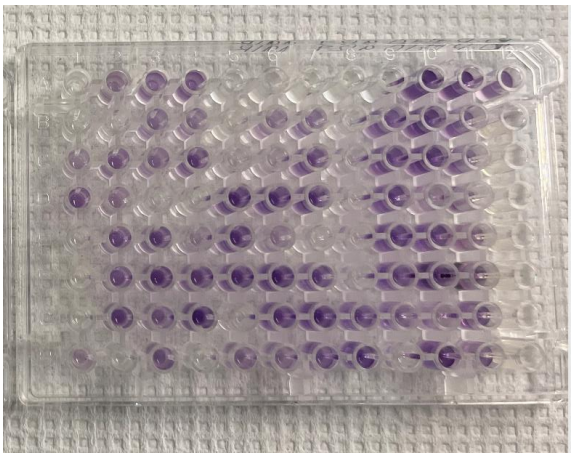
A)  B) 

FIGURE 5. Biolog assay of WT *E. coli* and *ΔgalK E. coli (*WT on the top (A) and *ΔgalR* on the bottom (B), reading from top left as A1).

Carbon Source Utilization Assay									
Condition	D- Gluconic Acid	D- Glucuronic Acid	Glucuronamide	D- Galactose	Methyl Pyruvate	L-Fucose	D-Fructose	Alpha D-Glucose	Acetic Acid
galS mutant	0.6645	0.7232	0.7158	0.7771	0.9627	0.7771	0.5856	0.3803	0.4101
galR mutant	0.6672	0.7315	0.7816	0.7402	0.5960	0.6999	0.5530	0.4802	0.7069
galK mutant	0.6053	0.6434	0.6195	0.1688	0.5251	0.6232	0.4916	0.4472	0.5092
Wildtype (WT)	0.5649	0.6547	0.6906	0.6955	0.4658	0.2460	0.5101	0.4997	0.5739

FIGURE 6. Color Gradient Table of Carbon Source Utilization from Biolog Plates for the *WT, ΔgalR, ΔgalS* and *ΔgalK* strains. Darker colors indicate higher absorbance and more electron transport activity in the wells. All the data is standardized to negative control. Numbers are absorbance measurements.

Chemical Sensitivity Assay						
Condition	Sodium Butyrate	Troleandomycin	4% NaCl	Tetrazolium Blue	Potassium Tellurite	pH 5
galS mutant	0.3566	0.3437	0.7766	2.8009	0.1145	0.8248
galR mutant	0.5488	0.2869	0.7289	0.1622	0.1218	0.6664
galK mutant	0.3658	0.1073	0.8822	2.8452	0.1448	0.4554
Wildtype (WT)	0.3737	0.3339	0.8789	2.8688	0.1734	0.2460

FIGURE 7. Color Gradient Table of Chemical Sensitivity from Biolog Plate for the *WT, ΔgalR, ΔgalS* and *ΔgalK* strains. Darker colors indicate higher absorbance and more electron transport activity in the wells. All the data is standardized to negative control. Numbers are absorbance measurements.

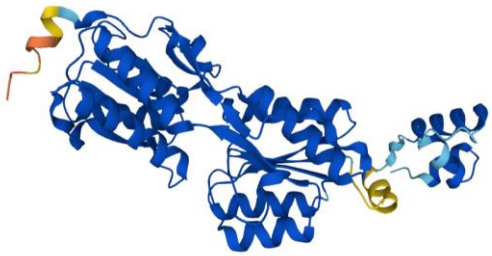


FIGURE 5. Protein ribbon structure of GalR protein from Alphafold. This protein was mutated in *ΔgalR E.coli* strain so that it was knocked. The GalR protein has a dimer structure and helix-turn-helix domains aka common DNA-binding domain that allow it to bind to DNA and regulate DNA expression.

Collectively, our data points towards the idea that *galR, galK* are essential genes and are required for galactose metabolism. *The E. coli galS* gene has been proven to be nonessential.

DISCUSSION

From our experiment, we were able to determine that *galR* and *galK* are essential genes, required for galactose metabolism, and that *galS* gene has been proven to be nonessential. With this knowledge, we have a further understanding of how *E. coli's* galactose metabolism works and how it connects and interacts with glucose metabolism. Prior to this paper, we only had a good understanding of the hierarchy between lactose and glucose metabolism for *E. coli* (1). However, now, we also have a better understanding of the hierarchy between galactose and glucose metabolism for *E. coli.* While we were also able to determine that *galR* and *galK* are essential genes for galactose metabolism, there is still much research that could and need to be done on these *gal* genes and how they work together and regulate the galactose metabolism. This paper gives a good stepping stone for further research that could give us more insights on how these genes affect other bacteria that may only use galactose as a carbon source. In conclusion, there is still much to be done to advance our knowledge about *E. coli* and its metabolic processes.

REFERENCES

1. Blaiseau, P. L., & Holmes, A. M. (2021). Diauxic Inhibition: Jacques Monod's Ignored Work. *Journal of the history of biology*, *54*(2), 175–196. https://doi.org/10.1007/s10739-021-09639-4

2. Baba, T., Ara, T., Hasegawa, M., Takai, Y., Okumura, Y., Baba, M., Datsenko, K. A., Tomita, M., Wanner, B. L., & Mori, H. (2006). Construction of Escherichia coli K-12 in-frame, single-gene knockout mutants: the Keio collection. *Molecular systems biology*, *2*, 2006.0008. https://doi.org/10.1038/msb4100050
3. Neidhardt, F. C., Bloch, P. L., & Smith, D. F. (1974). Culture medium for enterobacteria. *Journal of bacteriology*, *119*(3), 736–747. https://doi.org/10.1128/jb.119.3.736-747.1974
4. Lim, J. Y., Yoon, J., & Hovde, C. J. 2010. A brief overview of Escherichia coli O157:H7 and its plasmid O157. *Journal of microbiology and biotechnology*, *20*(1), 5–14.
5. Grenier, F., Matteau, D., Baby, V., & Rodrigue, S. 2014. Complete Genome Sequence of Escherichia coli BW25113. *Genome announcements*, *2*(5), e01038-14. https://doi.org/10.1128/genomeA.01038-14
6. Görke, B., and J. Stulke. 2008. Carbon catabolite repression in bacteria: many ways to make the most out of nutrients. Nat. Rev. Microbiol. 6: 613– 624
Traxler, M.. 2023. Lab 3: *E. coli* physiology project experimental design. Plant and Microbial Biology C112L
7. Adhya, S. 1987. The galactose operon, p. 1503–1512. In F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), Escherichia coli and Salmonella typhimurium: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
8. Geanacopoulos, M., & Adhya, S. 1997. Functional characterization of roles of GalR and GalS as regulators of the gal regulon. *Journal of bacteriology*, *179*(1), 228–234. https://doi.org/10.1128/jb.179.1.228-234.1997