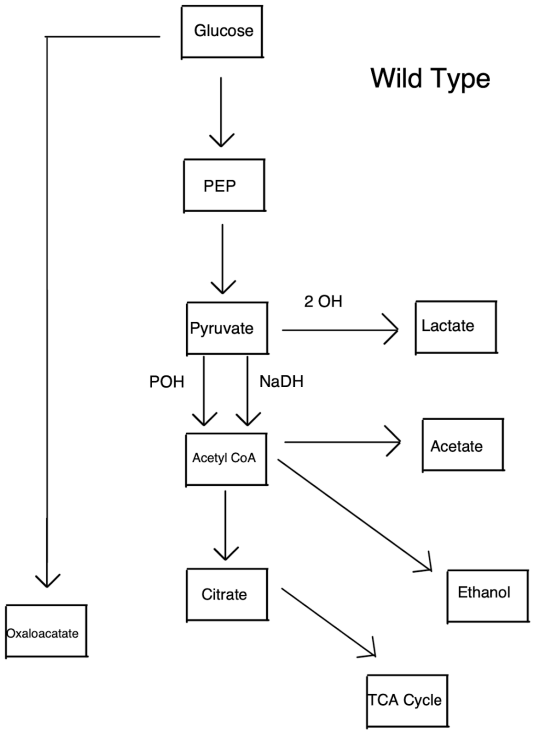
Introduction

E.Coli is a gram negative bacteria whose structure includes a rod shaped bacteria and is classified under the phylum Proteobacteria. E.Coli can grow in conditions with and without oxygen and is often found in the lower intestines of mammals but can be found in nature or vegetation. The genetics of the E.coli are particularly interesting as its genome consists of a singular circular DNA molecule. Despite this, the E.Coli genome consists of much variability and causes significant differences especially on the metabolism of the bacterium.On the topic of sugar metabolism, E.Coli has specific mechanisms that target a specific sugar and can be influenced by genetic manipulation.

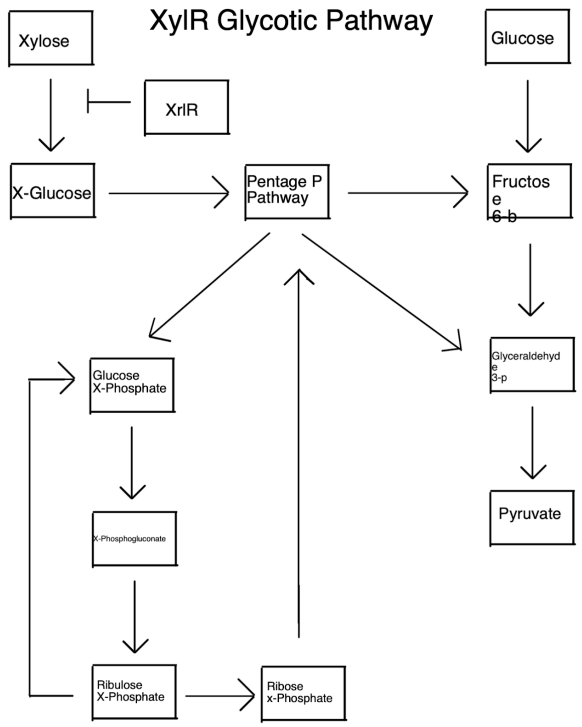
The reason for this experiment is to demonstrate the differences between the growth of E.Coli under different sugar conditions specifically Glucose and Xylose. To explicitly show this, we utilized two sugars, Glucose and Xylose in order to visualize the metabolism of Glucose before the Xylose. For this experiment, we had a Wild Type E.coli as the control and XylR mutant strain as the test subject to illustrate the discrepancies between a typical E.coli to a mutant strain.

The wild type strain E.Coli acts as the control of the experiment and a base for comparison to the other mutant strains. For the two sugars in question, Xylose and Glucose, the E.Coli has a particular method for metabolism. Glucose is targeted first by the E.coli since glucose is its primary carbon source and energy source. (Herz et al., 2017) E.Coli is particularly effective in metabolism under any condition since it can do so with or without Oxygen. After the glucose is converted, the Xylose gets targeted by the E.Coli. Xylose is converted into xylulose by the action of xylose isomerase. (Bañares et al., 2021)



The mutant strain XylR is a repressor in E.coli that is essential for the metabolism of sugars. It is a transcriptional regulator which means that it controls gene expression in xylose utilization. E.Coli needs specific enzymes to break down sugars which are on the Xyl operon.

Once Xylose is absent, the XylR acts as a repressor, binding to the operator sites and effectively ending gene expression. However, once Xylose is present, the protein will undergo a conformational change which promotes the metabolism of the sugar. (Phue et al., 2004)



Materials and Methods

Strain Table

Strain Name	Genotype
WT	F-, DE(araD-araB)567, lacZ4787(del)::rrnB-3, LAM-, rph-1, DE(rhaD-rhaB)568, hsdR514
crp	F-, DE(araD-araB)567, lacZ4787(del)::rrnB-3, LAM-, rph-1, DE(rhaD-rhaB)568, hsdR514Δcrp
xylA	F-, DE(araD-araB)567, lacZ4787(del)::rrnB-3, LAM-, rph-1, DE(rhaD-rhaB)568, hsdR514ΔxylA
xylR	F-, DE(araD-araB)567,

	lacZ4787(del)::rrnB-3, LAM-, rph-1, DE(rhaD-rhaB)568, hsdR514ΔxylR

Methods:

1. Grow overnight cultures of three *E.coli* mutant strains and one control wild type *E.coli* strain in 1 mL MOPS minimal medium with 0.1% glucose.
2. Prepare four Eppendorf tubes each with 1 mL MOPS minimal medium with 0.035% glucose and 0.075% xylose.

MOPS minimal medium:

Solution	**Amount for 1mL (uL)**	**[Final]**
10X MOPS Stock Solution	100	1 x
0.132 M K2HPO4 (Filtered 0.2 mm)	10	1.32 mM
40% Glucose (C source)	0.875	0.035%
40% Xylose (secondary sugar)	1.875	0.075%
1 M NH4CL	20	20 mM
autoclaved nano-H2O	867.25	
TOTAL	**1000**	

3. Measure the OD of each of the four overnight cultures of *E.coli*.
   For each strain:
   a. Add 4.5 mL of sterile PBS to a cuvette and blank using the spectrophotometer.
   b. Add 500 uL of the *E.coli* culture to the cuvette and measure OD.
4. Inoculate each Eppendorf tube containing medium with the four *E.coli* strains to obtain OD 0.05.
5. Measure growth curves of the four strains in Biolog.

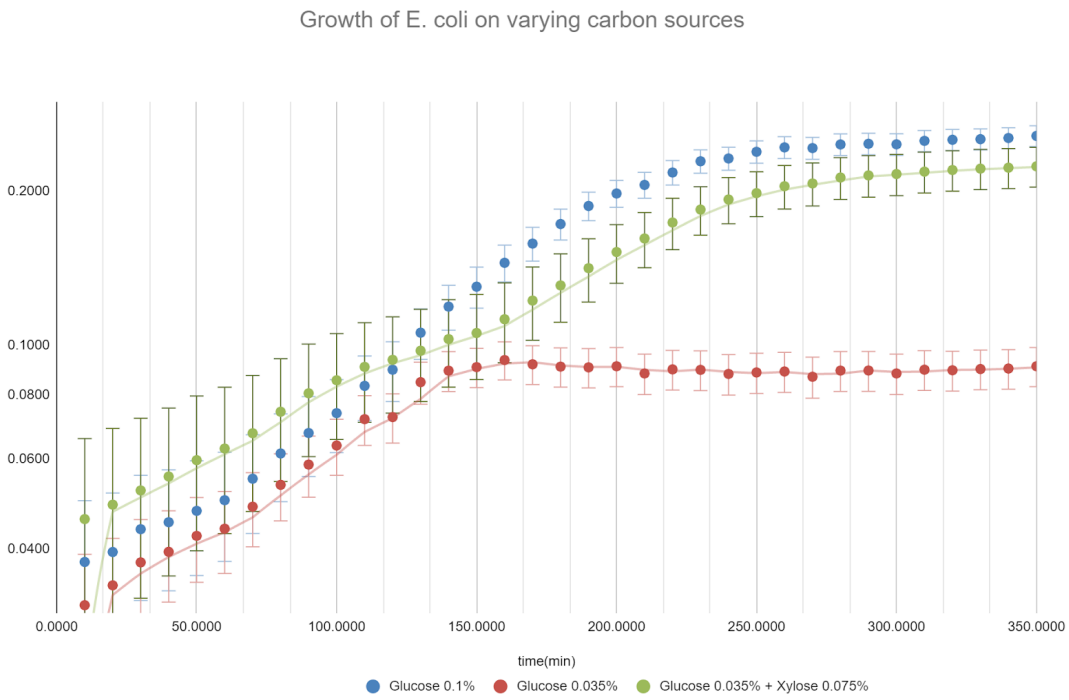
Results

***E.coli grown* on both glucose and xylose results in a diauxic shift**

E.coli cultured in the presence of both glucose and xylose was able to grow using both sugars as carbon sources, and experienced a diauxic lag during the switch to a new carbon source. In the presence of only glucose or only xylose, diauxic lag was not observed. *E.coli* experienced greater growth in the presence of higher concentration of glucose (0.1%) compared to lower concentration of glucose (0.035%). Growth in the presence of glucose and xylose achieved a similar level as a high concentration of glucose alone (Fig 3A). The shortest doubling time was

exhibited in the 0.1% glucose condition and the highest doubling time was exhibited during the diauxic shift in the presence of both glucose and xylose (Fig 3B).

A



B

	Glucose 0.1%	Glucose 0.035%	Glucose 0.035% + Xylose 0.075% - on Glucose	Glucose 0.035% + Xylose 0.075% - on Xylose	Glucose 0.035% + Xylose 0.075% - diauxic shift
Doubling Time (min)	69.039	75.696	90.301	98.683	171.147
Growth Rate Constant	0.0100	0.0091	0.0077	0.0070	0.0040

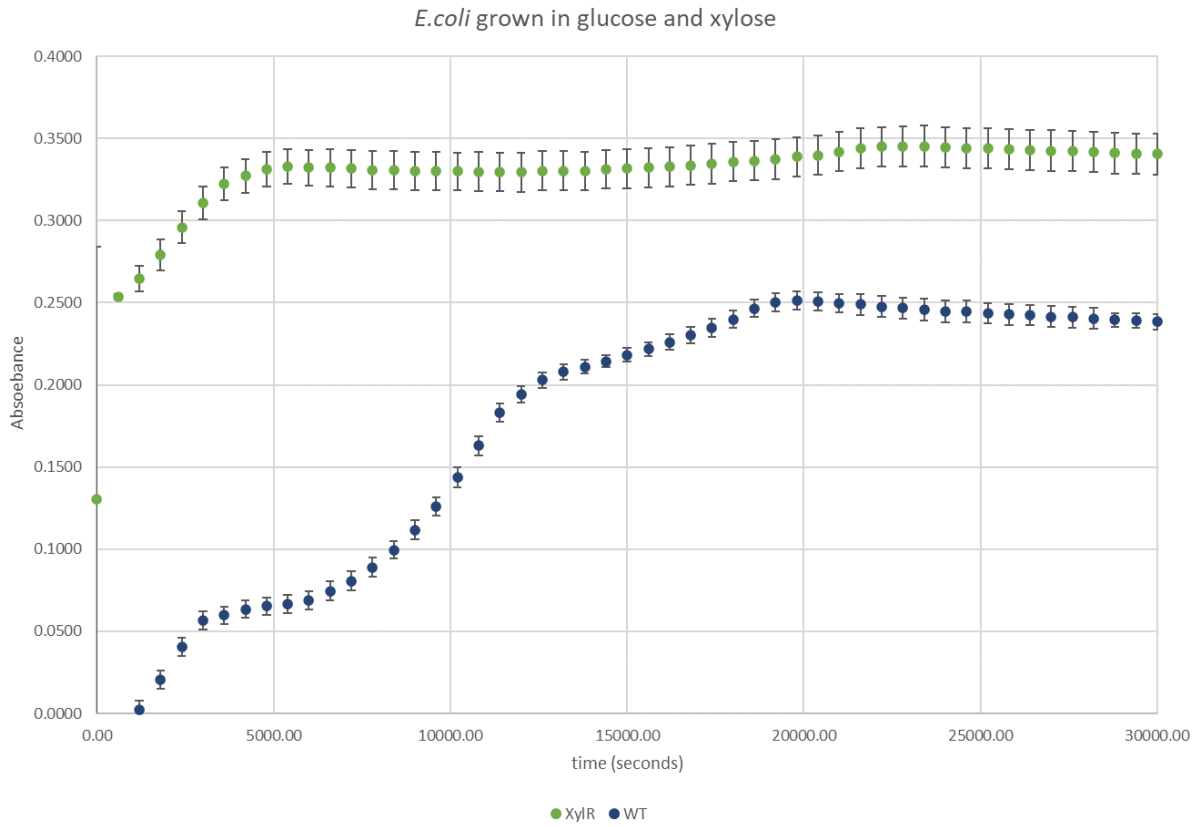
Figure 3: Growth curves (A) and doubling time (B) of wild type *E. coli* in the presence of varying carbon sources. *Cultures were grown with three conditions: 0.1% glucose, 0.035% glucose, and 0.035% glucose with 0.075% xylose. Data shown from 0 minutes - 350 minutes after incubation at 37C.*

Deleting xylR increase *E.coli* growth in the presence of both glucose and xylose

Compared to wild type *E. coli* grown in the presence of both glucose and xylose, ΔxylR *E.coli* exhibits increased growth while using glucose and xylose in comparison to the WT. The diauxic shift on the WT demonstrates the default consumption of the sugars. At the stationary phase, ΔxylR reached a slightly lower concentration compared to wild type (Fig 4A). ΔxylR had a

similar doubling time to wild type using glucose, but a lower doubling time compared to wild type using xylose (Fig 4B).

A



B

	WT - using glucose	WT - using xylose	WT - diauxic shift	ΔxylR
Doubling Time	87.596	160.860	442.143	4.843
Growth Rate Constant	0.0079	0.0043	0.0019	0.1431

Figure 4: Growth curves (A) and doubling time (B) of wild type and ΔxylR *E. coli* strains in the presence of glucose and xylose. *All cultures were grown with concentrations of both 0.035% glucose and 0.075% xylose. Data shown from 0 - 40000 seconds after incubation at 37C.*

Low absorbance High absorbance
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A **WT**

Blank	**L-Arabinose**	**N-Acetyl-D-**	**D-Saccharic**	**Succinic**	**D-Galactose**

0.2168	0.4868	**Glucosamine** 0.4974	**Acid** 0.6221	**Acid** 0.2145	0.2336
D-Serine 0.2130	**D-Sorbitol** 0.2812	**Glycerol** 0.5891	**L-Fucose** 0.5071	**D-Glucuronic Acid** 0.2687	**D-Gluconic Acid** 0.5772
D-Glucose-6-Phosphate 0.6142	**D-Galactonic Acid-y-Lactone** 0.6659	**D, L-Malic Acid** 0.6385	**D-Ribose** 0.8100	**Tween 20** 0.3739	**L-Rhamnose** 0.3681
L-Asparagine 0.6930	**D-Aspartic Acid** 0.5200	**D-Glucosaminic Acid** 0.6008	**1,2-Propanediol** 0.2769	**Tween 40** 0.8181	**a-Keto-Glutaric Acid** 0.7662
L-Glutamine 0.3758	**m-Tartaric Acid** 0.7577	**D-Glucose-1-Phosphate** 0.6077	**D-Fructose-6-Phosphate** 0.3468	**Tween 80** 0.7163	**a-Hydroxy Glutaric Acid-y-Lactone** 0.3653
Glycyl-L-Aspartic Acid 0.2812	**Citric Acid** 0.6881	**m-Inositol** 0.7073	**D-Threonine** 0.6869	**Fumaric Acid** 0.7249	**Bromo Succinic Acid** 0.7433
Glycyl-L-Glutamic Acid 0.2174	**Tricarballylic Acid** 0.6097	**L-Serine** 0.6006	**L-Threonine** 0.7487	**L-Alanine** 0.3105	**L-Alanyl-Glycine** 0.6460
Glycyl-L-Proline 0.3188	**p-Hydroxy Phenyl Acetic Acid** 0.3104	**m-Hydroxy Phenyl Acetic Acid** 0.6081	**Tyramine** 0.3589	**D-Psicose** 0.4876	**L-Lyxose** 0.5356
L-Aspartic Acid 0.1742	**L-Proline** 0.2819	**D-Alanine** 0.2115	**D-Trehalose** 1.1864	**D-Mannose** 1.1366	**Dulcitol** 0.7834
D,L-a-Glycerol-	**D-Xylose**	**L-Lactic Acid**	**Formic Acid**	**D-Mannitol**	**L-Glutamic Acid**

Phosphate 0.5718	0.3321	0.6110	1.0236	0.7035	0.1971
D-Fructose 0.6867	**Acetic Acid** 0.3689	**a-D-Glucose** 0.7747	**Maltose** 1.2700	**D-Melibiose** 1.0445	**Thymidine** 0.6358
a-Keto-Butyric Acid 0.8112	**a-Methyl-D-Galactoside** 0.3028	**a-D-Lactose** 0.7147	**Lactulose** 0.5942	**Sucrose** 1.1218	**Uridine** 0.5220
a-Hydroxy Butyric Acid 0.2897	**b-Methyl-D-Glucoside** 0.3488	**Adonitol** 0.7775	**Maltotriose** 0.7485	**2-Deoxy Adenosine** 0.7707	**Adenosine** 1.1435
Propionic Acid 0.6935	**Mucic Acid** 0.2874	**Glycolic Acid** 0.7092	**Glyoxylic Acid** 1.0084	**D-Cellobiose** 3.3587	**Inosine** 3.1171
Acetoacetic Acid 0.7597	**N-Acetyl-b-D-Mannosamine** 0.7167	**Mono Methyl Succinate** 0.5937	**Methyl Pyruvate** 0.6277	**D-Malic Acid** 0.8480	**L-Malic Acid** 0.7458
Glucuronamide 0.4759	**Pyruvic Acid** 0.6782	**L-Galactonic Acid-y-Lactone** 0.3847	**D-Galacturonic Acid** 0.6967	**Phenylethyl-amine** 1.2626	**2-Aminoethanol** 0.5776

B **Xyl R**

Blank 0.1545	**L-Arabinose** 0.2792	**N-Acetyl-D-Glucosamine** 0.3329	**D-Saccharic Acid** 0.5433	**Succinic Acid** 0.6321	**D-Galactose** 0.5482
D-Serine 0.5208	**D-Sorbitol** 0.5493	**Glycerol** 0.6225	**L-Fucose** 0.4547	**D-Glucuronic Acid** 0.6705	**D-Gluconic Acid** 0.5690
D-Glucose-6-Phosphate 0.7040	**D-Galactonic Acid-y-Lactone** 0.4205	**D, L-Malic Acid** 0.5055	**D-Ribose** 0.7204	**Tween 20** 0.1438	**L-Rhamnose** 0.1720

L-Asparagine 0.3548	**D-Aspartic Acid** 0.1615	**D-Glucosaminic Acid** 0.1646	**1,2-Propanediol** 0.1471	**Tween 40** 0.1559	**a-Keto-Glutaric Acid** 0.4055
L-Glutamine 0.2255	**m-Tartaric Acid** 0.2737	**D-Glucose-1-Phosphate** 0.6714	**D-Fructose-6-Phosphate** 0.7673	**Tween 80** 0.1345	**a-Hydroxy Glutaric Acid-y-Lactone** 0.1629
Glycyl-L-Aspartic Acid 0.6698	**Citric Acid** 0.1385	**m-Inositol** 0.1304	**D-Threonine** 0.2047	**Fumaric Acid** 0.4611	**Bromo Succinic Acid** 0.3530
Glycyl-L-Glutamic Acid 0.5868	**Tricarballylic Acid** 0.1623	**L-Serine** 0.6182	**L-Threonine** 0.3684	**L-Alanine** 0.4158	**L-Alanyl-Glycine** 0.5837
Glycyl-L-Proline 0.6189	**p-Hydroxy Phenyl Acetic Acid** 0.0951	**m-Hydroxy Phenyl Acetic Acid** 0.4404	**Tyramine** 0.3183	**D-Psicose** 0.2195	**L-Lyxose** 0.3514
L-Aspartic Acid 0.4958	**L-Proline** 0.2824	**D-Alanine** 0.4508	**D-Trehalose** 0.2712	**D-Mannose** 0.2487	**Dulcitol** 0.1982
D,L-a-Glycerol-Phosphate 0.6175	**D-Xylose** 0.3331	**L-Lactic Acid** 0.5429	**Formic Acid** 0.1538	**D-Mannitol** 0.1165	**L-Glutamic Acid** -0.0016
D-Fructose 0.2778	**Acetic Acid** 0.3302	**a-D-Glucose** 0.2658	**Maltose** 0.2544	**D-Melibiose** 0.2484	**Thymidine** 0.2349
a-Keto-Butyric Acid 0.2613	**a-Methyl-D-Galactoside** 0.4571	**a-D-Lactose** 0.1488	**Lactulose** 0.1823	**Sucrose** 0.1261	**Uridine** 0.0715
a-Hydroxy Butyric Acid 0.2856	**b-Methyl-D-Glucoside** 0.2812	**Adonitol** 0.1599	**Maltotriose** 0.2761	**2-Deoxy Adenosine** 0.2133	**Adenosine** 0.1983
Propionic	**Mucic Acid**	**Glycolic Acid**	**Glyoxylic**	**D-Cellobios**	**Inosine**

Acid 0.1875	0.5715	0.3362	**Acid** 0.3681	**e** 0.4425	0.4731
Acetoacetic Acid 0.2187	**N-Acetyl-b-D-Mannosamine** 0.2721	**Mono Methyl Succinate** 0.1895	**Methyl Pyruvate** 0.3997	**D-Malic Acid** 0.3851	**L-Malic Acid** 0.4311
Glucuronamide 0.0944	**Pyruvic Acid** 0.4178	**L-Galactonic Acid-y-Lactone** 0.1130	**D-Galacturonic Acid** 0.3916	**Phenylethyl-amine** 0.4009	**2-Aminoethanol** 0.4596

Figure 6: Heatmap of absorbance in WT (A), ΔxylR (B) grown with varying carbon sources in PM1 Biolog Plates.

The results on the absorbance heatmap are generated based upon the data found in our experiments and color coded accordingly. For instance, the darker red indicates low absorbance while the green color indicates higher absorbance. Particularly for the WT, we see more of a neutral absorbance with most of the colors being yellow while the ΔxylR demonstrates a range of colors with absorbance being evenly distributed. This information details that the WT is consistent with the data it produces and the absorbance while the ΔxylR shows that there becomes more variance amongst the absorbance once that happens.

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