

## Population growth and lag time assent in Lactose induced *Eschericia coli* Stains

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### ABSTRACT

Research has been done on the doubling time and population dynamics of *Eschericia coli* in multiple environments of lactose. As the percent of lactose increases and dextrose decreases in the Soy-broth, the *Eschericia coli* strains (lac+ and lac-) have longer lag times. The growth rate was measured by a Spectonic Genesys 2 spectrophotometer over 24 hours. Population estimates on growth was measured by colony counts on Soy-agar plates taken at time 0, 6, 18, and 24 hours. The *Eschericia coli* strain that lacked the lactose gene *B-galactosidas* (lac-) had similar absorbance patterns in 60%, 80%, and 100% lactose environments. The lac-strain in all lactose environments had higher absorbance readings than the lac+ *E. coli* strain, indicating a higher growth rate. The lac+ *E. coli* strain showed decreased absorbance as the percentage of lactose in its environment increased. It was concluded that the *E. coli* lac- strain grows better than the lac+ in all lactose environments. An extended stationary phase was indicated in both strains of *E. coli* beyond 24 hours of growth and needs to be confirmed with further testing.

Keywords: *β-galactosidase*, *Eschericia coli*, lag time

### INTRODUCTION

Intestinal gas, nausea, diarrhea and abdominal cramping are symptoms when the human body is not producing B-galactosidase. The body produces B-galactosidase in the fluids of the digestive track; the most common place in the body for these fluids to produce B-galactosidase is in the colon. (Jenny Harper, 2005) The B-galactosidase gene is induced when food consumed containing lactose are ingested. B-galactosidase breaks down the lactose into galactose and dextrose (Thomas, 1977). This helps the absorption of galactose and dextrose in the small intestines and then into the blood stream.

Lactose is a disaccharide, which means it is a two ring structure. The two sugar rings that make up lactose are the stereoisomers; Galactose and Glucose (dextrose). As compared to Dextrose being a monosaccharide (a single ring structure). This means there is twice the energy in lactose as there is in dextrose, if cells have the appropriate enzyme to break the bonds.

When *E. coli* replicates it goes through a series of growth phases. The first phase is known as the lag phase, when there is low growth detection due to low cell mass. During this phase the *E. coli* is producing the enzyme needed to utilize the substrate provided. Next, the log phase is an active growth stage. This phase lasts as long as there is cell division in abundant substrate. The stationary phase occurs when *E. coli* reaches growth equilibrium with its environment. The population of *E. coli* will fluctuate around the carrying capacity of the closed system once population density is achieved. When the waste acuminates to a toxic level and the amount of substrate is in decline the *E. coli* will experience a death phase. New research shows that there is an

extended stationary phase after an initial death process. In this phase the *E. coli* goes into survival mode, scavenging dead cell mass and mutating. This mutation of enzyme utilization allows the *E. coli* to live longer.

Research has been done on the doubling time and population dynamics of *Eschericia coli*. *E-coli* have been found to utilize lactose by using B-galactosidase. Jenny Harper tested the doubling time and population dynamics on 0%, 10%, 20% and 40% of lactose replacing dextrose in a growth media. This research project continued testing population dynamics on 0%, 60%, 80%, and 100% lactose replacing dextrose in the growth media. I also have assessed a strain of *E. coli* that does not have the capacity to produce B-galactosidase for lactose degradation. Instead of looking at the relationship between lactose environment and population dynamics, I tested the lag time and population dynamics.

From past research the lag times have not been constant with the environment of lactose. The lag time should be longest in the 100% lactose. Lag time refers to how long it takes the E-coli to utilize lactose instead of glucose.

### MATERIALS AND METHODS

Media Preparation:

Broth and agar media were prepared according to *E. coli* specification within Bergey's Manual (1957). All media were autoclaved at 121 degrees Celsius for 45 minutes. Agar plates were prepared aseptically within the biological safety cabinet once the agar had cooled to 70<sup>0</sup> C. Approximately 20 ml of melted agar

was poured per plate. Agar plates were refrigerated inverted to avoid condensation on the agar surface. Approximately 10 ml of broth was dispensed into test tubes. The amount of sugar in the Broth recipe was originally 0.250 grams of Dextrose. This amount in grams stayed the same at all times. Lactose was added to the recipe as Dextrose was taken out of the recipe. The amount of Dextrose decreased as the amount of Lactose increased.

#### Media Recipe:

The Difco media, Tryptic Soy Agar ingredients was add per liter. The ingredients include Trypton 7.5grams, Pancreatic Digest of Casein Soytone 2.5grams, Pancreatic Digest of Soy Meal Sodium Chloride 2.5 grams, and Agar 7.5 grams. The other media is Tryptic Soy Broth. Its ingredients are per fourth a liter. The ingredients include Tryptone 4.25grams, Pancreatic Digest of Casein Soytone 0.75grams, Pancreatic Digest of Soybean Meal **Dextrose** 0.250grams, Sodium Chloride 1.25grams, Dipotassium Phosphate 0.625grams, **Lactose**

#### Bacterial Cultures:

Bacterial cultures of *E. coli* and *E. coli lac-* were maintained weekly with a loop transfer to two separate broth test tubes. Both strains were incubated for 24 hours prior to experimentation to assure a fresh culture.

#### Population Growth Assessment:

Once a fresh 24 hour *E. coli* and *E. coli lac-* culture is prepared, they are assessed with the spectrophotometer. Spectrophotometer cuvettes were sterilized for 30 minutes under UV light. Two milliliters of broth was transferred to each of the 3 cuvettes. The *E. coli* strains were inoculated into their appropriate cuvettes with a loop transfer. The samples were assessed for absorbance every 2 minutes for 24 hours to indicate growth. A 0.1 ml sample was plated in triplicate on Soy-Agar at time 0, 6, 18 and 24 hours. The strains of *E. coli* were assessed in varying amounts of lactose in the broth recipe.

#### Population Evaluation:

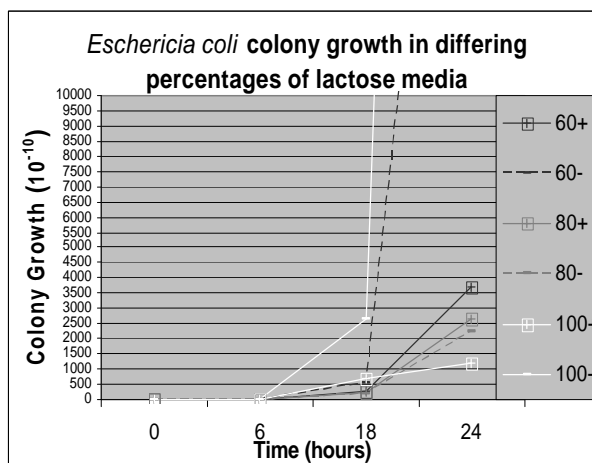
Colony forming units on soy agar plates were taken at time 0, 6, 18, and 24 hours. To see the colonies I had to do a serial dilution. Times 0, 6, 18, and 24 hours were all done at a different serial dilution. After each sample was spread the agar plates were then placed in 37 degrees Celsius for 24 hours of growth. The plates were placed upside down to reduce the moisture collection on the agar. Bacteria estimates were made by counting all colony forming units on all replicate plates at the appropriate dilution and producing an average number of colonies.

#### Gram Staining:

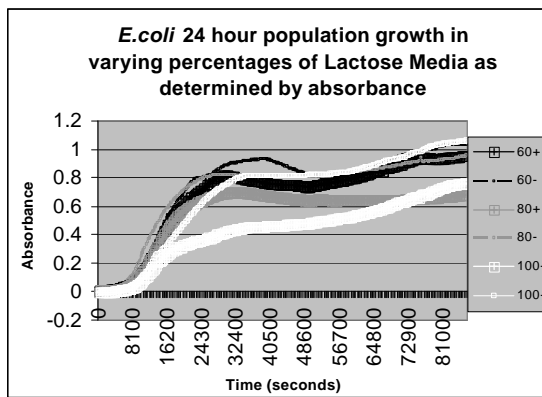
A Gram stain was done to restore confidence that the integrity of *E. coli* was intact and it was the only bacteria worked with in the duration of this experiment. Gram staining was done to help identify cell wall structure and morphology. A streak isolated colony of *E. coli* was sampled and placed on a slide. The colony on the slide was then flooded with Hucker ammonium oxalate crystal violet for sixty seconds, then rinse with tap water. The sample was then flooded with Gram's iodine solution for sixty seconds, and then rinsed with tap water. A decolorizing step followed with 95% ethanol. I allowed the ethanol to drip across the slide until the runoff was almost clear, then rinsed with tap water. The final stain of safranin was added to the sample for sixty seconds. I rinsed for the last time with tap water and blotted carefully not to disturb the stain. For a clear look at the bacteria I had to use oil on the immersion lens.

#### RESULTS

Sample	0 hours	6 hours	18hours	24hours
60+	0.001467	1.4133	258	3688
60-	0.0019	1.36	570.67	30900
60C	0	0	0	0
80+	0.0002	3.2267	245.33	2633
80-	0.0003	3.6267	197.33	2233
80C	0	0	0	0
100+	0.000033	3	696.7	1210
100-	0.000033	2.0267	2660	98667
100C	0	0	0	0



The graph above shows the colony growth of *E. coli lac-* and *lac+* in many different medias (60% lactose and 40% Dextrose, 80% Lactose and 20% Dextrose, 100% Lactose and 0% Dextrose). We can see that the 100% *lac-* had the most colony growth out of all the media. The 100% *lac+* seems to have the least growth. The growth for the *lac+* goes in order from, 60% being the most growth to 100% being the least.



The graph above shows the results from the spectrophotometer. This graph shows similar results with the colony growth assessment but more detail can be viewed with the continuous absorbance readings. In this graph you can see lac+ 100% has the least growth. After the 100%, comes the 80% followed by the 60% with the most growth for the lac+. The lac- *E. coli* is not as easy to see. The 80% starts out having the most growth with is shortest lag time, but then 60% lac- comes in with a large growth peak. At the end the 100% lac- has the highest growth out of all the Medias.

## DISCUSSION

The lag time of *Escherichia coli* increased as the percent of lactose was increased. The lac- strain had more growth then the lac+. This is not as expected because it is missing the gene to utilize the lactose. The lac- was put in the experiment as another control and this turned out not to be the case at all. This may have been because the *E. coli* mutated to survive, and somehow became better at utilizing lactose. There is indefinitely room for more studies in this area. One other option is that the lac- *E. coli* growth is that the company Carolina Biological Supply sent the enzyme  $\beta$ -galactosidase. In case of this then an electro freezes would help me despiser this.

The extended stationary stage was not experienced in the runs. This was because the run where only twenty four hours long. If the runs were ran for forty eight hour the extended stationary stage would have been experienced. New research show the there is an after stationary stage. The after stationary stage is when the *Escherichia coli* no longer has food to utilize and has to cannibalize for survival. At the stationary stage the *Escherichia coli* starts to have some of it's cells die and the absorbance line on a forty eight hour graph shows some of the cell dieing off, but then it also shows the growth going back up. When the growth goes back up, this is when the cells do what they can to survive by cannibalizing the other *E. coli*. The cells then grow and divide this is the extended stationary stage. The research is fairly new and leaves room for farther research.

The more lactose the longer lag time, the 100% Lactose and 0% Dextrose lac+ had the longest lag time. The 60% lac+ had the shortest lag time out of the lac+ runs. With this, I came to the conclusion that my results supported the Hypothesis.

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