

Influence of Lactose Utilization and Population Dynamics of *Escherichia coli* var. *mutabilis*

Jenny L. Harper

ABSTRACT

30-50 million American are lactose intolerant and suffer with intestinal gas, nausea, diarrhea or abdominal cramping when they eat foods that contain lactose. The digestive track is a microecosystem that contains numerous bacterial species, eight of which have been identified by Dr. Schaedler and his colleagues. This research was intent on investigating how one of these bacteria, *Escherichia coli* var. *mutabilis* (*E. coli*), utilized lactose when lactose was added into the bacteria's environment. *E. coli* was cultured in a Tryptic Soy Broth (TSB) containing 0%, 10%, 20% and 40% lactose (LTSB) in relation to the total amount of dextrose used in the media. Kinetics tests were run to find an approximate growth rate for the bacteria. Serial dilutions and population counts were performed from a 24-hour culture. The greatest amount of growth after 25 hours was found in the 0% LTSB and growth continued to decrease through the experiment with the least amount of growth found in the 40% LTSB. Statistical analysis of the population counts illustrated only the 20% lactose run had a significant P-value of 0.0048, therefore proving the null hypothesis incorrect-that bacteria can grow with lactose in the environment.

Keywords: *Escherichia coli* var. *mutabilis*, Lactase, Lactose, Lactose intolerance, and Population dynamics.

INTRODUCTION

Approximately 30-50 million people in the United States are lactose intolerant ("Digestive Diseases Statistics," 1994). Lactose intolerance is the inability to endure lactose-rich products because these subjects lack the enzyme lactase (Thomas, 1977). Lactase is an enzyme that can be found within the juices of the digestive track, specifically the colon. Lactase splits lactose into galactose and dextrose (Thomas, 1977), so that the nutrients from lactose-rich foods can then be absorbed by the small intestine into the blood stream. Of the millions who are lactose intolerant, most patients have in their bodies a small amount of the lactase enzyme. This enables them to utilize enzyme tablets that contain lactase (Thomas, 1977), before they eat milk products, and avoid symptoms such as bloating, cramps, intestinal gas, nausea, or diarrhea (Kemper, 1995). Small percentages of people totally lose the enzyme lactase and are unable to digest lactose.

The digestive track is a microecosystem containing numerous bacterial species, as many as 10^{11} bacteria/g of feces (Dewhirst, et al., 1999). All the microorganisms contribute to the health of the body in some fashion, whether it is to provide nutrients or to protect the body from pathogens. Altogether, the communities of enteric microorganisms contribute to the overall catabolism of substrates. Many organisms require very specific conditions and are influenced by limiting factors such as oxygen and carbon sources. The dominant organism is best adapted to this particular environment, and its metabolism alters the substrates and influences the environmental conditions. The

interconnections of these enteric populations are all part of the circle of life.

For many years scientists have believed that the gut contained an undefined mixture of different types and strains of bacterial that were not culturable (Bielke, et al., 2003). However, in the mid-1960s, Russell W. Schaedler and his colleagues isolated a certain selection of bacteria that include aerobic bacteria and oxygen-sensitive anaerobic bacteria. This selection was labeled "Schaedler flora," and it contained eight bacteria: *Escherichia coli* var. *mutabilis*, *Streptococcus faecalis*, *Lactobacillus acidophilus*, *Lactobacillus salivarius*, group N *Streptococcus*, *Bacteroides distasonis*, a *Clostridium* sp., and an extremely oxygen-sensitive fusiform bacterium (Schaedler, et al., 1965: cited in Bielke, et al., 2003).

This research project will investigate the microflora competition in a lactose-rich environment. The objectives of the study were as follows: first, to identify the doubling times for the bacteria used; and second, to culture bacteria in 0%, 10%, 20%, and 40% lactose-rich (in relation to the amount of dextrose used) media. *Escherichia coli* var. *mutabilis* (*E. coli*) will be used in this project.

MATERIALS AND METHODS

Bacteria strain and media. *Escherichia coli* var. *mutabilis* (*E. coli*) was obtained from Carolina Biological Supply Company (Burlington, NC) in a dehydrated form. After rehydration, the *E. coli* was placed into an incubator (Fisher Scientific Incubators)

at 37°C for 24 hours, then transferred into Tryptic Soy Broth (TSB).

E. coli was cultured in TSB containing 0%, 10%, 20% and 40% lactose (LTSB) in relation to the amount of dextrose used in the media. Broth and agar (TSA) were autoclaved (Tuttnauer-Brinkmann Autoclave-Steam Sterilizer) at 121°C at 15 lbs per pressure for 15 minutes. Before medium was inoculated with bacteria, test tubes and plates were set out to room temperature.

NOTE: Medium was made without using a premix due to the requirement of controlling the specific amounts of lactose (Difco Laboratories, Detroit, MI).

Kinetics tests were run to find the approximate growth time for *E. coli*. Plastic cuvetts were sterilized in a hood under UV light (Nuair Biological Safety Cabinets, Plymouth, MN) for one hour. Two milliliters of 0% LTSB were added to two cuvetts, then 0.01 ml of a 24-hour culture was added to one cuvette; the other is a controlled blank. The cuvetts were placed in the Spectronic Genesys 2 Spectrophotometer and the Kinetics test was run for 24-hours in a 37°C water bath. A sigmoid curve was then produced (Figure 1) to give an approximate growth rate for the bacteria.

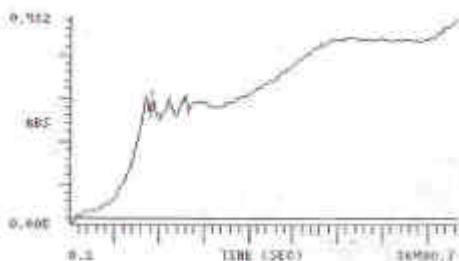


Figure 1. 24 hour spectrophotometer graph of *Escherichia coli* in a 0% LTSB.

Dilutions and population counts. From a 24-hour culture a serial dilution was performed with a disposable sterile pipette to inoculate a test tube containing 10 ml TSB. The tube was vortexed and 0.1 ml was removed with a pipette to inoculate a second 10 ml TSB tube. Population counts were taken after six dilutions for the 0% LTSB, after eight dilutions for the 10% LTSB and after ten dilutions for both the 20% and 40% LTSB. After the final dilution, 0.3 ml was removed with a pipette to be added to three 20 ml TSA plates (0.1 ml per plate), spread and placed into a 37°C incubator for 24 hours. This procedure was repeated every four hours as determined by the sigmoid curve produced in the kinetics test.

After 24 hours in the incubator, population counts were taken on the plates made. Petri dish bottoms were divided into quarters; counts were then made in

a single section and the multiplied by four. The approximate totals from the three plates per time period were then averaged to get an estimation of growth per hour.

Both the dilutions and population counts were repeated for each percentage (0%, 10%, 20%, and 40%) of lactose.

Statistical Analysis. The computer program Sigma Plot was utilized during data analysis. Regression Wizard at the 3rd Parameter (ANOVA: Partial F test) was enabled to obtain statistical information (Equation 1).

$$N(t) = \frac{a}{1 + e^{-rt}} \quad (\text{Eq. 1})$$

RESULTS

Bacterial growth was found on all plates for each percentage of lactose (Table 1). The greatest amount of growth after 25 hours was found in the 0% LTSB and growth continued to decrease through the experiment with the least amount of growth found in the 40% LTSB.

Evidence of significant growth in the 0% LTSB is illustrated in Table 1 by large amount of “Too Numerous To Count” (TNTC) boxes filled. The average of colonies at 25 hours was 456 colonies, at 26 hours the average colony count was 549 and at 27 hours the average colony count was 2,645 colonies. These tallies were greater than 30-300 colonies per plate; this is showing more dilutions are needed.

Table 1. *E. coli* colony population counts at each time period and at each percentage of lactose.

Time (Hr)	Plate	0%	10%	20%	40%
25	1	257	8	129	58
	2	721	7	188	128
	3	670	22	128	261
26	1	2484	775	650	333
	2	3012	1043	431	454
	3	2440	753	371	857
27	1	2056	1066	853	492
	2	TNTC	1184	988	748
	3	TNTC	1440	1388	TNTC
28	1	TNTC	1780	1964	TNTC
	2	TNTC	1424	1932	TNTC
	3	TNTC	904	1888	TNTC

Table 2. P-values for each percentage of lactose.

% of lactose	P-value
0%	0.9973
10%	0.374
20%	0.0048
40%	0.4.392

Statistical results by Sigma Plot produced a significant P-value for the medium containing 20% lactose. The other percentages produced insignificant results (Table 2). A sigmoid curve was produced that combined each of the percentages of lactose (Figure 2).

Figure 2 combines the four different growth curves for each of the percentages of lactose. The solid line is the 0% LTSB, a true sigmoid curve. The dotted line is the 10% LTSB, a much smaller sigmoid curve. The dash-dot-dot-dash line is the 20% LTBS, the bacteria is taking much longer to grow and has not yet hit its peak (K-value). The dash line is the 40% LTSB, illustrating a much slower growth rate than the other environments.

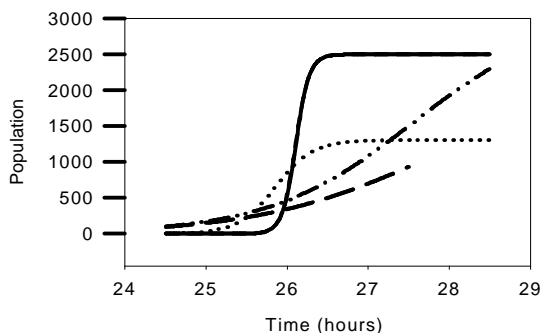
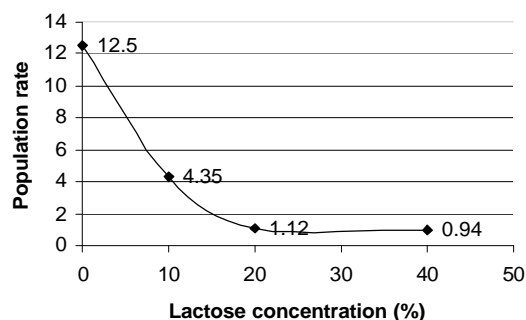
**Figure 2.** Sigmoid curve for *E. coli* grow in each of the different environments (0%, 10%, 20%, 40% LTSB).**Figure 3.** Population rate versus lactose concentration.

Figure 3 illustrates there was growth throughout the experiment. When zero percent lactose was added to the environment of the bacteria, there was the most growth. As the concentration of lactose was increased the growth rate of the *E. coli* decreased.

DISCUSSION

Ten to seventeen percent of the United States population (2000 US Census) is lactose intolerant; this is why it is important to better understand how the gut works in relation to the food people consume and how the bacteria work in breaking it all down so the body can utilize the much-needed nutrients.

There have been numerous studies on lactose malabsorption, but hardly any on actual intolerances of lactose. This research is just beginning to help researchers gain a better understanding of how one bacterium, *Escherichia coli* var. *mutabilis* (*E. coli*), found in the gut (Schaedler, et. al., 1965) reacts to the addition to lactose into its environment.

When lactose was added into the environment, as a certain percentage of the total sugar-source, it was evident the *E. coli* was still able to function and grow (Table 1). As population counts were made, some of the plates had too many colonies to get a specific number of colonies thus were considered TNTC. This is a lack of accuracy in data collection and could be resolved if more dilutions were completed.

The higher the percentage of lactose in the TSB, the slower the *E. coli* grew (Figure 3). This could be a result of the *E. coli* not converting over to the lactose as a sugar source; the bacteria were still utilizing the dextrose more completely even as the amount available decreased.

Because this project is a building block for future research, some improvements can be made:

- 1) Use of the spectrophotometer to obtain a sigmoid curve for each percentage of lactose. This would gain insight into where samples should be taken and then dilution runs should be made to acquire population counts.
- 2) Continue with the same methodology and increase the amount of lactose added to the environment (60%, 80% and 100% LTSB). It would be important to compare and contrast results of the 0% LTSB and 100% LTSB. By completing this step, a better understanding of how *E. coli* utilizes the lactose can be obtained.
- 3) To ensure purity of the culture, it would be recommended that a Gram-Negative staining should be performed throughout the entire testing process.
- 4) Multiple testing should be completed. By completing multiple tests of each experiment, averages can be calculated to counter-balance irregularities, bad techniques and niches.

The influence of lactose on *E.coli* is that it slows the growth rate of the bacteria. However, because the study did not include an environment in which there was 100% lactose, it has not been discovered if *E. coli* can completely exist in a lactose-only environment. *E. coli* might better survive in a lactose-only environment if there were other types of bacteria that could assist in breaking down the lactose so the *E. coli* could better utilize the products and have a more positive growth rate.

ACKNOWLEDGEMENTS

This work was supported in part by the Natural Science Department of McPherson College.

I would like to thank Dr. Karrie Rathbone, Associate Professor of Biology, McPherson College, McPherson, KS, for her guidance, insight, humor and encouragement throughout the project. In addition to the other professors in the department for their feedback and support, I would like to thank Dr. Jonathan Frye, Associate Professor of Biology, for his patience and guidance with the statistical analysis.

I would also like to thank Dr. Bryan Larsen, Dean of Research, Department of Microbiology, Des Moines University, Des Moines, IA, for his guidance in the experimental design, and Ramahi B. Sarma-Rupavtarm, graduate student, Department of Civil and Environmental Engineering, MIT, Cambridge, MA, for his help with background research.

LITERATURE CITED

- "2000 US Census." Information & Research Services. Internet Staff (Population division). 25 January 2002. US Census Bureau. 23 March 2005 <http://www.census.gov/main/www/cen2000.html>.
- Bielke, L.R., A.L. Elwood, D.J. Donoghue, A.M. Donoghue, L.A. Newberry, N.K. Neighbor, and B.M. Hargis. 2003. Approach for Selection of Individual Enteric Bacteria for Competitive Exclusion in Turkey Poults. *Poultry Science* 82(9): 1378-1382.
- Difco Manual: Dehydrated culture media and reagents for Microbiology. 10th ed. Detroit, Michigan: Difco Laboratories, 1984.
- "Digestive Diseases Statistics." National Institute of Diabetes and Digestive and Kidney Diseases, National Institute of Health. National Digestive Diseases Information Clearinghouse. August 1999. National Institute of Health. 4 Nov 2004 <http://digestive.niddk.nih.gov/statistics/statistics.htm>.
- Dewhirst, F.E., C.C. Chien, B.J. Paster, R.L. Ericson, R.P. Orcutt, D.B. Schauer, and J.G. Fox. 1999. Phylogeny of the Defined Murine Microbiota: Altered Schaedler Flora. *Applied and*

- Environmental Microbiology* 65(8): 3287-3292.
- Kemper, D.W. 1995. *Healthwise Handbook: A Self-Care Manual for You*. Edition 12. Healthwise, Incorporated, Boise, ID. Page 262.
- Schaedler, R.W., R. Dubos, and R. Costello. 1965. Association of germfree mice with bacteria isolated from normal mice. *Journal of Experimental Medicine* 122:59-66.
- Thomas, C.L. 1977. *Taber's Cyclopedic Medical Dictionary*. Edition 19 F.A. Davis Company, Philadelphia, PA. Page I-41, L-6.