A Study of the Relationship Between Acidity and Bacteria Common in the Human Intestine

Dhruv Nalla

Abstract

In today's age, companies are producing more and more carbonated and sweetened beverages. The pancreas and stomach are responsible for breaking down the compounds in these drinks using acid, and drinking too much soda may cause pain or indigestion. But is it possible that acidic compounds could be a breeding ground for more serious issues, even including cancer? About 60-70 million people in the U.S. suffer from intestine-related diseases, including colon and intestinal cancer. A common cause of these cancers is Escherichia coli, a rod-shaped, coliform bacterium that, when introduced to a host, lives in the intestine. This study aims to find a relationship between the acidity of an E.coli culture and the number of E.coli cells present in that culture, incubated under similar temperatures sustained in the human body, in a period of three days. To successfully carry out this study, 10 Petri dishes were marked in pairs of two, marked down with their corresponding level of acidity. The dishes were marked in halves such that 4 trials could be made, and the pH of the dishes ranged from 2 (very acidic) to 11 (very basic). A serial dilution was used to achieve these levels, and a streaking technique was used to achieve accurate data with the cultures. The agar plates were incubated at 37 degrees Celsius, and after 4 days were taken out and examined. The zone of inhibition was measured for each culture. For more basic pHs, there was a larger zone of inhibition, while there was little to no zone of inhibition for the acidic cultures. This is significant to demonstrate a relationship between the acidity of an E.coli culture and the number of E.coli cells present in that culture, as the more acidic the culture is, the less inhibited the growth of the cells became.

Introduction and What We Know

Think back to the last time you had a can of soda or even a glass of orange juice; there might be a whole culture of bacteria growing inside of you disguised as a mild stomach ache. Although your pancreatic cells are likely to take care of the bacteria, an excessive amount of liquid containing acid may lead to much more serious problems. In the contemporary landscape of health research, the growing concern over the rising incidence of gastrointestinal (GI) cancers, particularly those affecting the colon, has become a critical area of investigation. Epidemiological studies consistently highlight the prevalence of colon cancer, placing it among the leading causes of cancer-related morbidity and mortality worldwide. While lifestyle factors such as diet, physical activity, and obesity have been implicated in the development of colon cancer, the intricate interplay between dietary choices and the microbiome within the gastrointestinal tract adds a layer of complexity to our understanding. The gut microbiome, a diverse community of microorganisms residing in the digestive tract, has emerged as a pivotal player in maintaining gut health. Disruptions in the balance of this microbial ecosystem have also been associated with an increased risk of colorectal cancer, with certain bacterial species, including E.coli, gaining attention for their potential role in the initiation and progression of colorectal tumors. The current research extends its focus beyond the immediate effects of acidic compounds on E. coli cultures, offering a glimpse into the broader implications for the gut

microbiome and, by extension, colorectal health. Clinical observations and molecular studies have identified specific strains of E.coli, such as enterotoxigenic E.coli (ETEC), as potential contributors to inflammation and DNA damage in the colon. Chronic inflammation, a hallmark of many gastrointestinal disorders, including inflammatory bowel disease (IBD), has been linked to an increased risk of colorectal cancer. Understanding how acidic environments influence the growth and behavior of E. coli in the context of the gut microbiome adds a layer of complexity to our comprehension of the multifactorial etiology of colorectal diseases.
Moreover, recent advancements in cancer research emphasize the importance of personalized medicine and targeted interventions. Understanding the significance of how acidic environments impact the dynamics of E. coli growth provides a stepping stone toward developing tailored therapeutic strategies.

Materials and Methods of Experimentation
E. coli K-12 from Carolina Biological Supply provided a standardized strain, offering consistency in the study. The use of vinegar and ammonia allowed for the creation of a diverse range of acidity levels, mimicking conditions encountered in dietary choices. The Petri dish, incubator, and agar collectively provided a controlled environment for the observation of E. coli under varying acidity conditions. Moreover, the incorporation of gloves, pipettes, measuring cups, measuring flasks, a pH reader, and a marker emphasized the importance of maintaining aseptic conditions and precise measurements throughout the experiment. The methodology encompassed a systematic series of steps designed to shed light on the impact of acidity on E. coli growth. The preparation of agar with Petri dishes ensured a suitable growth medium, and the serial dilution of vinegar facilitated the creation of a nuanced spectrum of acidity levels. The subsequent steps involved heating, streaking, and adding acidity to the Petri dishes, with meticulous attention to maintaining uniformity during the process. The marked dishes were then incubated at 37 degrees Celsius for four days, simulating the conditions within the human body. Following incubation, the measurement of zones of inhibition provided quantitative data, which was further evaluated, graphed, and charted (see “Experiment and Data”).

Experiment and Data
Following the incubation of the Petri dishes, the observed data revealed nuanced insights into the relationship between acidity levels and the inhibition of Escherichia coli (E.coli) growth. Notably, certain trials exhibited challenges, with either the absence of a clear zone of inhibition or deformities that hindered accurate interpretation. However, despite these variations, the presence of inhibition zones was consistently discernible in at least one of the trials. Moreover, a noteworthy trend emerged, indicating that as acidity levels decreased, the size of the inhibition zones also diminished. This pattern provided a foundation for asserting a correlation between acidity levels and the extent of bacterial growth inhibition. Nevertheless, it is crucial to acknowledge that the data for the neutral pH level was inconclusive, posing limitations on the establishment of a direct correlation in this specific instance.

Figure 1

Figure 2

The samples at pH level 2 had little to no zones of inhibition

Figure 3

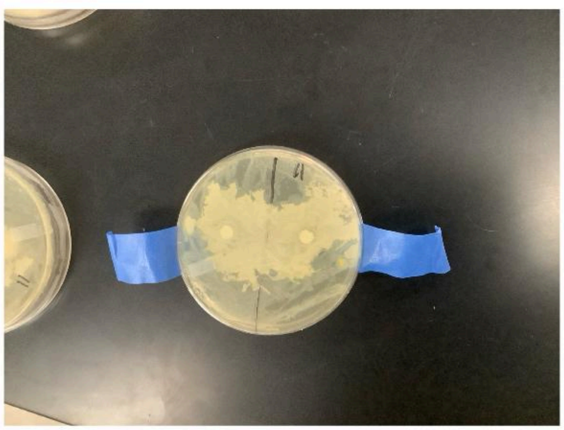
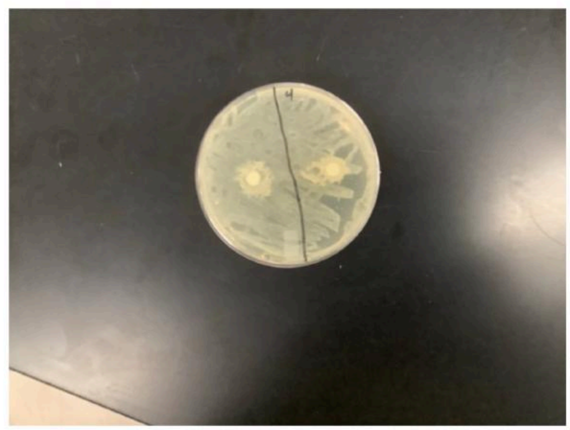


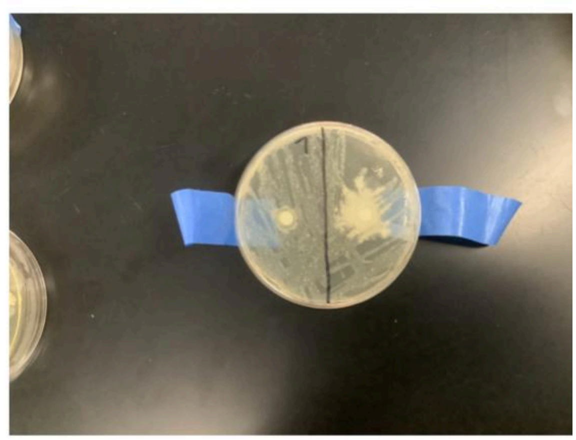
Figure 4



The samples at pH level 4 showed an increase in inhibition length

Figure 5

Figure 6



The samples at pH level 7 showed little increase in inhibition length, but still noticeable and large

Figure 7

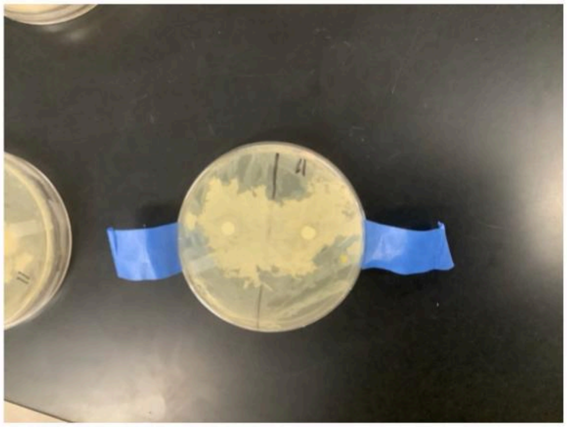
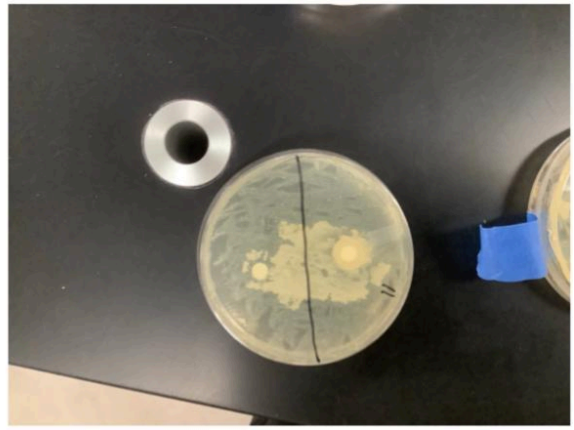
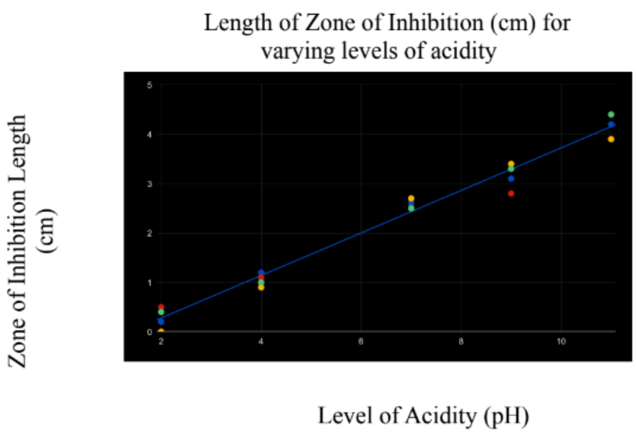


Figure 8



The largest inhibition zones can be seen at pH level 11

Figure 9



Each dot represents 1 trial (4 trials per level of acidity)

In concluding the experiment, it is evident that some plates presented inconclusive results. However, a discernible and consistent trend underscored a correlation between acidity levels and *Escherichia coli* (E. coli) growth. The general pattern indicated that lower pH levels corresponded to reduced inhibition zones, suggesting a potential inhibitory effect of acidity on bacterial growth. This aligns with the initial hypothesis and adds a layer of understanding to the intricate dynamics between acidic environments and bacterial behavior. Despite the inconclusive data for the neutral pH level, the overall trend observed in the plates supports the hypothesis that acidity plays a role in modulating bacterial growth. These findings contribute valuable insights to the broader context of gastrointestinal health research, offering a basis for further exploration and potential applications in preventive strategies against bacterial-induced diseases.

Application and Further Research

The implications of this research extend beyond the laboratory, holding potential applications that could influence the practices of beverage companies. The evidence generated through this project serves as a compelling argument for beverage companies to reconsider and potentially lower the quantity of sweeteners and carbonated beverages in their products. By doing so, there is a prospect of mitigating the number of individuals suffering from gastrointestinal diseases and, notably, colon cancer. This application aligns with public health initiatives aimed at reducing the prevalence of conditions associated with dietary choices. The research provides a scientific basis for advocating changes in beverage formulations, contributing to a broader conversation about the impact of dietary components on gastrointestinal health. In envisioning future research endeavors, the data obtained from this study opens avenues for more nuanced investigations. A logical extension would involve revisiting the experiment but with a novel approach—using hydrochloric acid instead of vinegar. This adjustment seeks to simulate the acidic conditions within the human stomach more accurately. By replicating the stomach's acid environment, researchers can anticipate more precise data, enhancing the reliability and applicability of the findings. This future research direction holds promise in unraveling the specific dynamics between stomach acidity and bacterial behavior. It also presents an opportunity to delve deeper into the potential effects of various acidic environments on Escherichia coli (E.coli) growth, providing a more comprehensive understanding of the interplay between dietary components, stomach conditions, and gastrointestinal health. Such efforts contribute to the continuous evolution of scientific knowledge, guiding future interventions and preventive measures in the realm of digestive health.

References

[1] Nouri, R., Hasani, A., Shirazi, K. M., Alivand, M. R., Sepehri, B., Sotoodeh, S., Hemmati, F., & Rezaee, M. A. (2022). Escherichia coli and Colorectal Cancer: Unfolding the Enigmatic Relationship. Current pharmaceutical biotechnology, 23(10), 1257–1268. https://doi.org/10.2174/1389201022666210910094827

[2] Roberts, M. (2022, February 27). E. coli gut infection linked to bowel cancer. BBC. Retrieved November 21, 2023, from https://www.bbc.com/news/health-51626946

[3] Callaway, E. (2022, August 16). E. coli strain linked to cancer in mice. Nature. Retrieved January 11, 2024, from https://www.nature.com/articles/nature.2012.11211

[4] Davenport, L. (2020, March 3). Strain of E coli linked to about 5% of colorectal cancer. Medscape. Retrieved January 11, 2024, from https://www.medscape.com/viewarticle/926121

[5] Bonnet, M. (2024, February 16). Clinical cancer research. Retrieved January 11, 2024, from https://aacrjournals.org/clincancerres/article/20/4/859/78872/