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Article

Ramifications of natural and artificial sweeteners on the gastrointestinal system

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SUMMARY

As the clamor for healthier diets increases, many alternatives to glucose, the standard sugar, are coming into focus. We aimed to determine whether artificial sweeteners are harmful to the human microbiome by investigating two different bacteria found to be advantageous to the human gut, *Escherichia coli* and *Bacillus coagulans*. To measure the variation in bacterial growth relating to each sweetener, we mixed different sweeteners – glucose and stevia as natural sweeteners, and sucralose and acesulfame potassium as artificial sweeteners – with agar on which the bacteria would be growing. Then, both *E. coli* and *B. coagulans* were placed on separate agar plates and allowed to incubate for 24 hours and 96 hours, respectively. A dramatic reduction in bacterial growth was observed for the agar plates containing the two artificial sweeteners in comparison to the two natural sweeteners. Using an ANOVA test, we were able to demonstrate that when comparing each of the four combinations of artificial sweeteners to natural sweeteners, there was a significant difference in perceived growth. This led to the conclusion that both artificial sweeteners inhibit the growth of the two bacteria and warrants further study to determine if zero-sugar sweeteners may be worse for the human gut than natural sugar itself.

INTRODUCTION

As people become more interested in lowering sugar and caloric intake, artificial sweeteners are becoming increasingly popular alternatives to natural sweeteners (1). Natural sweeteners, like honey and maple syrup, tend to be higher in calories and sugar. Artificial sweeteners, in contrast, are lower in sugar and calories while still providing a sweet taste, appealing to people globally (2). In 2022, the global artificial sweetener market reached 7.2 billion USD, and the market continues to rise (3). With such an expansion in the market, it is necessary to question how these new sweeteners could impact human health for the worse, rather than for the better as they may be advertised. Recent studies have shown that there are adverse health effects associated with using artificial sweeteners, particularly concerning gut health (4). They can also cause headaches, an increased risk of cancer, and weight gain (5).

Before modern chemistry, natural sweeteners were the only option for people, however today it is simple to create a new sweetener in a lab. For example, sucralose, an artificial sweetener, is synthesized from sucrose where three of the hydroxyl groups of the natural sugar are replaced with three chlorine groups. (6). A second artificial sweetener, acesulfame potassium, is created by simply combining acetoacetic acid and potassium. Due to how new these sweeteners are, research is consistently being gathered on how safe for human digestion they truly are. Our study aimed to determine the biological effects of artificial sweeteners in comparison to natural sweeteners on the human gastrointestinal system as modeled by two types of bacteria, *Escherichia coli* and *Bacillus coagulans*. These two bacteria were chosen as they have both been seen as a vital part of the microbiome. *E. coli* is one of the many bacteria which help with the digestion of food and is one of the few members of the microbiome capable of living with oxygen. Due to this, they can consume oxygen from the gut, allowing other microorganisms to live in an environment where they too can grow with little oxygen, and provide additional support in digesting food (7). Our second bacteria, *Bacillus coagulans*, is beneficial to the gut because it is one of the few probiotics able to survive in harsh conditions. This means that both bacteria help alleviate stomach distress as well as general digestion problems (8). Our research addressed whether statements made in the past few years about artificial sweeteners and their repercussions on health were facts, or myths.

We hypothesized that the bacteria exposed to the artificial sweeteners - sucralose and acesulfame potassium - would exhibit less bacterial growth than the bacteria exposed to natural sweeteners - glucose and stevia. The hypothesis was drawn based on research such as that done by Aparna Shil, where it was discovered that artificial sweeteners inhibited growth in *Enterococcus faecalis*, one of the many inhabitants of the microbiome. (5) If there was in fact less bacterial growth in our findings, this would signify that the artificial sweeteners were inhibiting the growth of bacteria found to be beneficial and necessary to the gastrointestinal system and human's ability to digest food and are therefore harmful for health. We found that the bacteria cultures plated on agar containing artificial sweeteners grew significantly less colonies than those plated on agar plates containing natural sweeteners. This was true of both bacteria types. This implies that the microbiome of the gastrointestinal system may be negatively affected by artificial sweeteners, as the growth of beneficial bacteria was inhibited by these sweeteners.

RESULTS

To accomplish this investigation, we used two different bacteria, *E. coli* and *B. coagulans*, and four different sweeteners, sucralose, acesulfame potassium, glucose, and stevia. We grew each type of bacteria on separate agar plates containing one of the four different sweeteners. We also included a control group, which had no sweetener. Following incubation, bacteria were photographed and compiled in forms that were sent out to individuals at Williamston High School Math and Science Academy, and each participant was asked to give each plate a rating on that scale from 1 to 10 identifying how covered in bacteria the plate appeared to be **(Figure 1).**

Looking at the bacteria growth in the petri dishes, there was a clear difference between the growth of bacteria plated in natural sweetener and the growth of bacteria plated in artificial sweetener, as both acesulfame potassium and sucralose displayed zero bacterial development **(Figure 2).** Both the *E. coli* and *B. coagulans* grown on Petri dishes with artificial sweeteners had significantly less growth than those grown with natural sweeteners for all combinations given ($p < 0.05$). There was significantly more *E. coli* growth for stevia than there was for ace K ($p < 0.001$, one-way ANOVA). This is one of many scenarios in which the artificial sweetener inhibited the growth of *E. coli* more than natural sweeteners. Likewise, there was significantly more *B. coagulans* growth for glucose than there was for sucralose ($p < 0.001$, one-way ANOVA). All eight comparisons followed the same trend. The mean values for *E. coli* growth had the most perceived growth at 5.41 ± 2.03 and stevia following close behind at 4.58 ± 0.78, with Ace K and sucralose displaying considerably less growth at

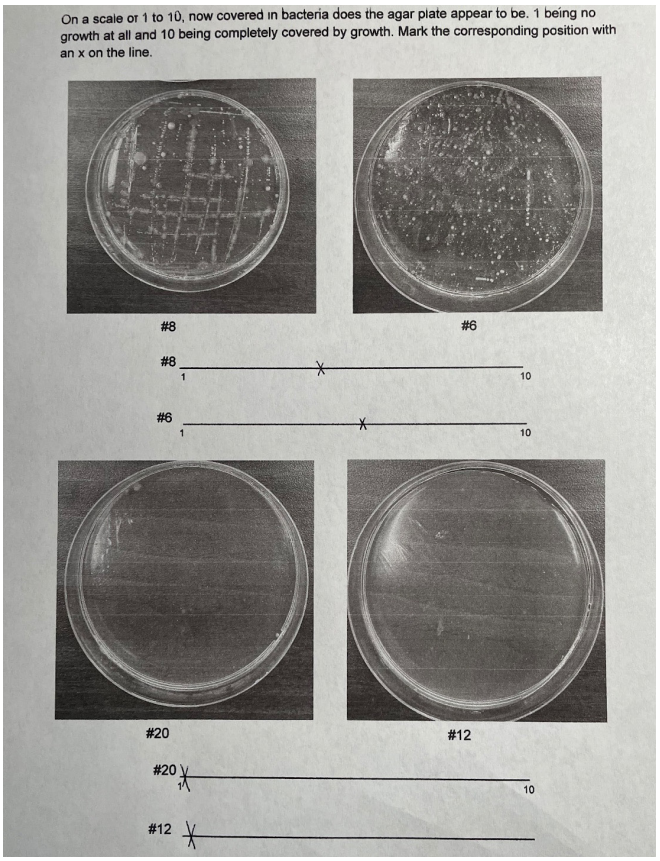

Figure 1. Representative form for bacterial growth. A completed form filled out by a student with perceived bacterial growth for each of the four pictures displayed. Each student rated bacterial growth on 20-40 plates, with each plate being rated a total of 100 times. Images displayed here all have *B. coagulans* and a combination of stevia or Ace K in a random order on the page.

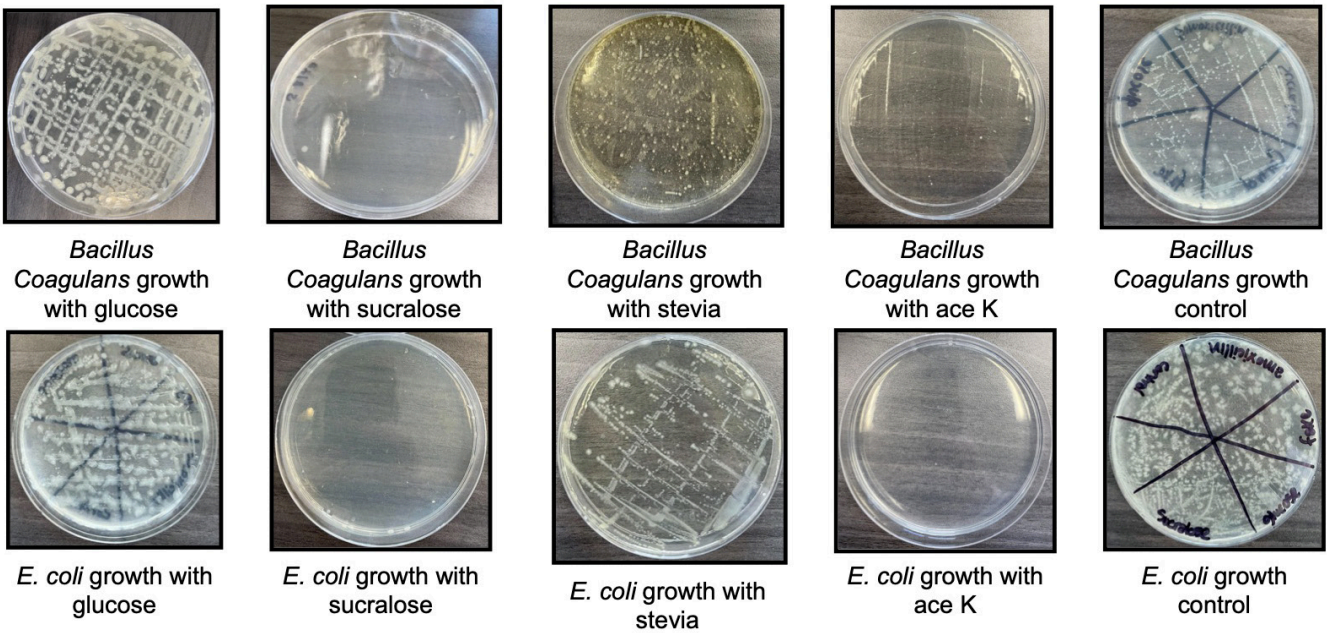

Figure 2. Representative images of the average bacterial growth on agar plates. Images display the agar plates with varying degrees of bacterial growth as well as both types of bacteria, *E. coli* and *B. coagulans* after incubation at 37°C for 24 and 96 hours, respectively. Bacterial growth was inhibited by both types of artificial sweeteners as $p < 0.001$.

1.05 ± 0.50 and 1.10 ± 1.07 respectively **(Figure 3)**. The same trend can be seen in the *B. coagulans* data in which glucose has growth of 6.28 ± 1.48 while stevia follows with 5.45 ± 1.76, and just as before, the two artificial sweeteners having the least growth with Ace K at 1.01 ± 1.34 and sucralose at 0.55 ± 0.28 **(Figure 4).**

DISCUSSION

We sought to determine if there was a significant negative effect of using artificial sweeteners on the growth of gut bacteria. We hypothesized that the plates mixed with artificial sweeteners would have less bacterial growth than the plates mixed with natural sweeteners. Our data supports this hypothesis, as there was a significant difference in the perceived mean bacterial growth between the natural and artificial sweeteners. We concluded that the artificial sweeteners sucralose and Ace K inhibited the growth of both species of gut bacteria supported by the statistically significant differences seen. Further, this also suggests that overall gut health is harmed by artificial sweeteners. Healthy gut bacteria, along with immune cells, provide the necessary environment to ward off viruses, harmful bacteria, and fungi. Artificial sweeteners alter the natural microbiome which is harmful to overall gut health. This could potentially cause irritable bowel syndrome, constipation, heartburn, and bloating. Such symptoms caused by artificial sweeteners reinforce our findings of their negative effects on gut health (9).

Our findings can be used for further research on the effects of artificial sweeteners on gut bacteria. One way that this further research could take place would be through comparing the different sweeteners that people use in their everyday life, such as artificial Sweet'N Low or Splenda, to natural table sugar rather than comparing the pure sweeteners. This way people would know which of the sweeteners offered in their typical coffee shop are the best for their gut health. Additionally, with this improved knowledge, knowing more about the harms of artificial sweeteners will allow for humans to be able to create new forms of sweeteners that are not as harmful to the gastrointestinal tract, while also helping those who want to cut back on their calories and/or sugar intake.

A limitation of our research was that we only used two species of gut bacteria. There are several other species of gut bacteria aside from the two used in this experiment; however, these were either too expensive or unable to be purchased for personal use. Only using two different strains of bacteria may not apply to the whole human microbiome. However, the results provide evidence to support our hypothesis. Another limitation of this study was that only two types of artificial or natural sweeteners were used. The FDA has approved five different artificial sweeteners: saccharin, aspartame, acesulfame potassium, sucralose, and neotame, all of which could have been used in this research. Other natural sweeteners include honey, pure maple syrup, agave nectar, etc. In this experiment, only two of each type of sweetener were used due to time and the limitations of the school year. While it would have been ideal to test all these sweetener and bacteria combinations at the same time, the structure of our experiments did not allow it.

Another part of our research that we changed from the standard was the way we collected data. The typical approach to collecting data would be through counting colonies of the bacterial growth, however we did not use this method, as it would have taken more time than we had in our one-hour class period. Had we used this procedure, it would be necessary to split data collection over a series of several days, and considering bacteria grows and dies rapidly, the results would not be consistent. While the survey result may introduce unnecessary subjectivity, we viewed it as the superior option given our circumstances and concluded through using several different people to fill out the form, subjectivity would be reduced. Even with these limitations

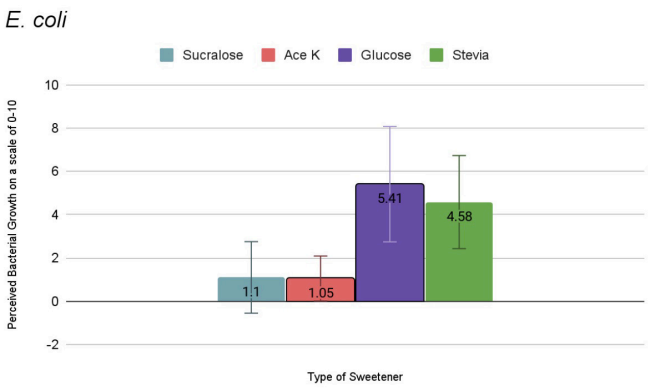

Figure 3. Mean perceived bacterial growth for *E. coli* for all types of sweeteners. *E. coli* was incubated at 37°C for 24 hours before measurement. Glucose and stevia display growth at 5.41 and 4.58 out of 10, respectively, and the two artificial sweeteners, Acesulfame potassium (Ace K) and sucralose, at 1.10 and 1.05 out of 10. Error bars present standard deviation.

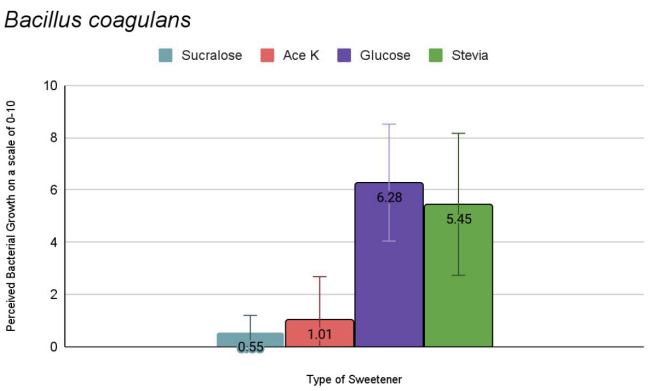

Figure 4. The mean perceived bacterial growth for *B. coagulans* for all types of sweeteners. *B. coagulans* were incubated at 37°C for 96 hours before measurement. Glucose and stevia display growth at 6.28 and 5.45 out of 10, respectively, and the two artificial sweeteners, Ace K and sucralose, at 0.55 and 1.01 out of 10. Error bars present standard deviation.

and room for procedural improvements, our findings provide helpful conclusions that are relevant for overall wellness and can be used for future research regarding the adverse effects of artificial sweeteners on gut health.

MATERIALS AND METHODS

Experimental setup and media preparation

We tested four different sweeteners, two natural, glucose and stevia, and two artificial, sucralose and acesulfame potassium, along with a control group. For the initial stage of our research there were three groups: sucralose, glucose, and a control group. The control group was plain agar with neither of the sweeteners mixed in. We began by melting premade nutrient agar (Carolina Biological Supplies) over a Bunsen burner. In each sterile petri dish, we mixed 10mL of the liquefied agar with one gram of the specific sweetener being tested, based on a previous experiment, until it had fully dissolved, and then poured it into an agar dish and waited until it had solidified, as modeled by previous studies (10). This concentration was chosen for its proportionality to the concentration of the sweetener in common sugary beverages (10). Ten of each type of plate (control, glucose, and sucralose) were created. After the agar solidified, the plates were covered with their corresponding lid and placed in a fridge for storage.

E. coli and *B. coagulans* culturing and scoring

We used the quadrant streaking method with an inoculating loop to spread *E. coli* evenly around the agar plate (10). Once all 30 plates had bacteria on them, we placed them in the incubator at 37°C, upside down for 24 hours (10).

Following this 24-hour period, we removed the plates from the incubator, and pictures were taken of each plate, all under the same conditions, ensuring that no glare was evident. To quantify the data, we hand delivered forms to 10 randomly selected students. These students were chosen from the 89 Math and Science Academy (MSA) members at Williamston High School. Each form contained images of the 30 plates, with four pictures on each page, and underneath each picture, a 10 cm scale, labeled 0 and 10 on each end. At the top of the page, we provided instructions to identify how covered in bacteria growth the plate appeared to be with an x on the line. The study was a double-blind study as neither the student filling out the form nor we knew which type of sweetener was on a particular plate.

Recording the data involved using a ruler to measure where a student had made their x along the line for the corresponding picture. This was recorded in a spreadsheet and combined with the other nine students' rankings for that same picture. Once all of these were recorded, we found the mean and standard deviations for all the ratings of each type of sweetener.

This process was repeated using *B. coagulans*. However, the *B. coagulans* incubated for 96 hours rather than 24 hours, as its growth was slower. A new batch of ten students was selected from the MSA without replacement, and the data was recorded in the same way.

For the second round of testing, the artificial sweetener used was Ace K, while stevia served as the natural sweetener. There was no control group used since we considered the control group from the initial round as a control basis for the two new sweeteners. The same process was repeated as before using these new sweeteners.

Statistical analysis

Following the data collection of all types of sweeteners and bacteria, we compared the results with one another. To find the comparison between all four combinations for each of the two types of bacteria, we ran a one-way ANOVA statistical test to have one set of comparisons to observe. This was done simply through entering each of the 100 rankings (10 plates with 10 rankings each) for each of the four separate types of sweeteners: glucose, sucralose, stevia, and ace K, into a computer program which would run the ANOVA test. This gave us both HSD values and *p*-values for each of the comparisons. We were then able to compare artificial sweeteners and natural sweeteners side by side by looking at examples such as the level of significant difference in *E. coli* growth between plates plated with sucralose and plates plated with stevia.

While we collected data for the control group, these numbers were not used when calculating the ANOVA statistical test as we believed their inclusion took away from the focus of our study. The sole purpose in including a control group was to show that the bacteria - E. coli and B. coagulans - were able to grow on the Petri dishes given the conditions that we used, which was found to be true.

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