Effect of caffeine and artificial sweetener on the growth and biofilm formation of Lactobacillus acidophilus.

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Abstract

Caffeine is an addictive substance that is commonly used as an additive in various food products. It has been proven that regular consumption of caffeine can lead to caffeine addiction. Furthermore, once the body has gotten used to drinking a certain amount of caffeine, the same amount of caffeine does not produce the same potent effects. Drinking large quantities of caffeine for a sustained period of time has serious repercussions on mood, attention span, memory, and processing speed due to changes in receptors in the brain and neurotransmitter release. Artificial sweeteners are also commonly used in food items and can lead to health issues. Artificial sweeteners such as Splenda are not recognized by the body and as such cannot be broken down into glucose to be used as ATP. The main ingredient in Splenda is sucralose and sucralose does not get broken down or stored by the body. *Lactobacillus acidophilus* (LA) is a gram-positive biofilm-forming resident bacteria that is found in the human mouth, intestine, and vagina. The biofilm forming capability of LA enables it to colonize areas on the surface of the intestine and survive the adverse conditions of the various bodily regions it lives in. Furthermore, forming a thick biofilm on bodily surfaces prevents colonization and invasion of opportunistic and pathogenic bacterial species. The objective of this study is to examine how artificial sweeteners such as Splenda and caffeine affect the growth and biofilm formation of LA. To determine the effect of caffeine on LA growth, varying concentrations of caffeine were added to a flat bottom 96 well microtiter plate containing LA stock culture in 100µL of MRS media. After 48 hours of incubation, LA growth and biofilm formation were observed using a spectrophotometer. The method used to detect biofilm formation was the crystal violet biofilm assay. The method used to detect LA growth was the spectrometer readings of blanks vs experimental groups. Under the crystal violet assay procedure, after 48 hours of LA culture in the experimental and control groups, the non-adhered LA bacteria was washed and removed, and the remaining bacteria was stained by crystal violet. The crystal violet stain was released with 100% ethanol and was measured via spectrophotometer. All data represented triplicate runs and the results for this experiment was inconclusive for the effect of caffeine and Splenda on biofilm formation as none of the wells contained biofilm. The results for the bacterial growth however, indicated that both Splenda and caffeine had more LA growth than the control samples.

Introduction

Lactobacillus acidophilus (LA) is a gram-positive resident bacteria that can be found in the human mouth, intestine, and vagina. It is commonly used as a commercial probiotic. LA's biofilm forming capabilities enable it to colonize body surfaces preventing opportunistic and pathogenic bacteria from invading host tissue (Jebur, 2010). Past studies have found that sucralose, which is the main ingredient in Splenda can inhibit *Lactobacilli* growth (Ruiz-Ojeda, 2019). Plain caffeine was found to limit *Lactobacilli* growth as well (Sales, 2020). This study was designed to examine if Caffeine and Splenda has an effect on growth and biofilm formation of LA in varying concentrations of Splenda and Caffeine.

Objectives

1. To examine the effect of Caffeine on LA growth and biofilm formation
2. To examine the effect of Splenda on LA growth and biofilm formation
3. To determine the effect of various concentrations of Caffeine and Splenda on LA growth and biofilm formation

Procedure

Effect of caffeine and Splenda on *Lactobacillus acidophilus* growth

A pipette tip full of LA glycerol stock was placed inside 3 mL of MRS media and incubated at 37℃ for 24 hours. Once culture was grown the 3 mL of inoculated media was added to 50 mL of fresh MRS media and was incubated at 37 ℃ for 48 hours. 100 µL of inoculated MRS media was then added to a 96-well flat bottom microtiter plate in triplicate. An additional 100 µL of fresh non-inoculated MRS media was added to the wells. In the control groups the additional MRS media did not contain additives. For the Splenda and caffeine test wells varying concentrations of Splenda and caffeine were added respectively to each plate in triplicate. Concentrations of Splenda and caffeine were increased in increments of 125 mg with the lowest concentration being 125 mg and the highest being 750 mg. LA growth was measured using absorbency data from spectrophotometer readings read at 450 nm. The data was then analyzed using a paired sample t-test to test for significant differences between control and experimental groups.

Effect of caffeine and Splenda on *Lactobacillus acidophilus* biofilm formation

A pipette tip full of LA glycerol stock was placed inside 3 mL of MRS media and incubated at 37℃ for 24 hours. Once culture was grown the 3 mL of inoculated media was added to 50 mL of fresh MRS media and was incubated at 37 ℃ for 48 hours. 100 µL of inoculated MRS media was then added to a 96-well flat bottom microtiter plate in triplicate. An additional 100 µL of fresh non-inoculated MRS media was added to the wells. In the control groups the additional MRS media did not contain additives. For the Splenda and caffeine test wells varying concentrations of Splenda and caffeine were added respectively to each plate in triplicate. Concentrations of Splenda and caffeine were increased in increments of 125 mg with the lowest concentration being 125 mg and the highest being 750 mg. The Crystal Violet Assay was preformed to observe biofilm formation. The dyed and alcohol filled wells were then placed in a spectrophotometer and read under the 450 nm wavelength. Analysis was not preformed.

Materials

Bacteria
Lactobacillus acidophilus

Media
200 ml of MRS broth

Statistical Analysis
Paired Sample T-test

Plates and Equipment
96 well plate
Multichannel pipette
500ml flask or container
Inoculating loop
Bunsen burner
Incubator
UV-V spectrometer

Reagents
Splenda
Caffeine power
Crystal Violet
DI water
90-95% Ethanol

Results

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Caffeine Control	0.9294	N/A	0.7444	0.6015	0.3724	0.7190
Splenda Control	1.4872	N/A	1.7609	2.4870	1.6144	1.3992
Caffeine	0.3238	0.3463	0.4293	0.5312	0.3138	0.5875
Splenda	0.1878	-0.2415	0.0346	1.0230	0.2041	0.1034

Table 1. Average difference of test in comparison to respective controls The average absorbency (nm) of test groups for caffeine and Splenda were compared to the average control for these two substances. The caffeine control category average was taken by subtracting the average absorbency data of the test group to that of a blank group that only contained caffeine and fresh non-inoculated MRS media. The Splenda control category average was taken by subtracting the average absorbency data of the test group to that of a blank group that only contained Splenda and fresh MRS media. Significant difference between controls and test groups using paired sample t-test at p=<0.05.

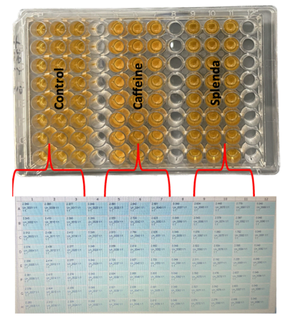

Figure 1. Absorbency data measured in nm for Group 3 Test Sample The top figure depicts one of the 96-well flat bottom microtiter plates loaded with a control, caffeine, and Splenda sample. The measurements at the bottom are the absorbency data measured in nm of the test samples.

Discussion

This experiment was not able to examine how Splenda and caffeine effect biofilm formation as the bacterial samples for all experimental groups did not form biofilm. More refined protocol is needed to procure biofilm. It is possible that the glycerol stock of LA was too old or the specific strain of LA tested was not able to form biofilm. More testing is needed to determine the effects of Splenda and caffeine on biofilm. This experiment found that increased concentrations of caffeine and Splenda increase LA growth whereas other studies have found the opposite to be true.

Conclusions

1. Caffeine and Splenda had a significant effect on LA growth
2. The results for the effect of caffeine and Splenda on biofilm growth was inconclusive as the test samples did not contain any biofilm
3. More refined protocol is needed to encourage LA biofilm formation

References

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