Journal of
Emerging Investigators

# *Bacteroides thetaiotaomicron* Growth Numbers are Unchanged in the Presence of Yogurt

Tina Phan, Pooja Patel, Stephanie Wallace, Lauren O'Keefe, Veronica Arseneault, Liam Gilligan, Riley Panneton, Kathleen Mungai, Katelyn St. Louis, Yaa Konama Pokuaa, Kimberly A. Gonzalez*

Lowell High School, Lowell, MA 01852, United States

*Corresponding author at: Tel: +1 978 934 8900. Email address: kgonzalez@lowell.k12.ma.us

**Summary**

**The microbiome is composed of microorganisms that prime the human immune system for proper functioning and aid in energy harvest and biomolecule production. Disruptions to the microbiome have been linked to the development of certain diseases such as diabetes and obesity. Recent phylogenetic analysis has shown that obese patients have an imbalance in the two major phyla, the Firmicutes and the Bacteroidetes that compose the gut microbiome. The obese gut has higher levels of Firmicute bacteria when compared to Bacteroidetes, which have reduced numbers. This is important because many probiotic foods and supplements are produced using bacteria that fall into the Firmicute phylum. This study explored whether or not Fage plain total 0% Greek yogurt, which contains live and active bacterial cultures belonging to the Firmicute phylum, could decrease the numbers of *Bacteroides thetaiotaomicron*, an organism found in the human gut that belongs to the Bacteroidetes phylum. *B. thetaiotaomicron* was grown in the presence of Fage plain total 0% Greek yogurt containing live and active cultures, and with re-pasteurized Fage plain total 0% Greek yogurt. ANOVA analysis indicated no statistically significant reduction in the numbers of *B. thetaiotaomicron* when grown in either yogurt condition. This study can be used to guide people in dietary measures meant to keep beneficial Bacteroidetes in the gut.**

**Received:** March 9, 2016; **Accepted:** October 25, 2016; **Published:** December 29, 2016



## Introduction

The microbiome of humans contains an estimated 100 trillion microbial cells as well as an estimated quadrillion viruses (3). It is responsible for energy harvest, the breakdown of indigestible carbohydrates, the production of important biological molecules, and, most importantly, proper immune system development. Microbiome development begins at birth, and by eleven months of age, infants have their own unique microbiome. The microbiome can be influenced by genetics, as shown in a study conducted by Benson *et al.* (2). In their study, different breeds of mice were crossbred and 645 fecal profiles were sequenced, with results showing 64 preserved taxonomic phyla. Variation in the amounts of these bacterial groups were analyzed using SNP markers that identified 18 host quantitative trait loci (QTL) groups, which were attributed to the variation in the microbiomes of the mice. Microbiome colonization can also be affected by life events such as antibiotic usage, food consumption, and environmental exposures (3).

Most microbiome-host interaction occurs in the gut of humans, and as such, the gut is heavily colonized by a variety of microorganisms (7). Phylogenetic analysis has shown that bacterial organisms belonging to the Firmicutes and the Bacteroidetes phyla dominate the human gut. More importantly, these analyses have shown that a disruption of the microbiome is been linked to chronic diseases such as obesity, inflammatory bowel diseases, and diabetes (3). Studies have indicated that in the obese gut, the ratio of Bacteroidetes to Firmicutes is altered. Obesity is linked to higher levels of *Lactobacilli* (a Firmicute) species and lower levels of Bacteroidete species in the gut (6). A study performed by Armougom *et al.* showed the average number of Bacteroidetes and Firmicutes in twenty lean individuals was 1.35E+10 and 2.16e+10, respectively, whereas twenty obese individuals had 3.76E+09 and 1.37E+10, respectively (1), showing reduced numbers of Bacteroidetes in the obese gut. This led us to question whether the consumption of probiotic foods, such as yogurts, could adversely alter the gut microbiome, because many probiotic foods and supplements contain organisms that belong to the Firmicutes phylum (5). To explore this possibility, cultures of *B. thetaiotaomicron,* a member of the Bacteroidetes phylum and inhabitant of the human gut, were grown under two conditions: a) in the presence of Fage plain total 0% Greek yogurt with live and active bacterial cultures; and b) in the presence of re-pasteurized Fage plain total 0% Greek yogurt. We hypothesized that the

*B. thetaiotaomicron* numbers would be reduced when grown with the Fage plain Greek yogurt containing live and active bacterial cultures. ANOVA statistical analysis revealed that there was no significant decrease ($p$ = 0.90) in the numbers of *B. thetaiotaomicron* regardless of whether or not it was grown with Greek yogurt that contained bacteria. These findings are important because they indicate that eating yogurt that contains live and active bacterial cultures may not reduce the numbers of beneficial Bacteroidetes species in the gut.

## Results

The number of colony-forming units per mL (CFUs/mL), a measure of the number of viable bacteria cells, was calculated for *B. thetaiotaomicron* growing a) in thioglycolate broth; b) in the presence of Fage plain total 0% Greek yogurt containing live and active bacterial cultures; and c) in the presence of re-pasteurized presence of Fage plain total 0% Greek yogurt. Using the Real Statistics Data Analysis Resource Pack for Excel (8), the mean CFUs/mL, sample variance, and standard deviation were calculated (**Table 1**). The mean CFUs/mL of *B. thetaiotaomicron* growing in thioglycolate broth was 23,473,333 after 24 hours of incubation at 35°C starting from a 0.5 McFarland standard (**Figure 1**). The mean CFUs/mL of *B. thetaiotaomicron* growing with Fage plain total 0% Greek yogurt containing live and active bacterial cultures was 29,846,667 after 24 hours of incubation at 35°C starting from a 0.5 McFarland standard (**Figure 1**). The mean CFUs/mL of *B. thetaiotaomicron* growing with re-pasteurized Fage plain total 0% Greek yogurt was 37,233,333 after 24 hours of incubation at 35°C starting from a 0.5 McFarland standard (**Figure 1**).

Since there were three groups being tested, an analysis of variance (ANOVA) was the appropriate statistical test to use. ANOVA testing produced a $p$ of 0.90, with $\alpha = 0.05$, showing that there was no statistical difference among the mean CFUs/mL of *B. thetaiotaomicron* growing in the three conditions. ANOVA testing was verified by a Levene's test. The Levene's test calculated $p$ = 0.55, showing that the variance of the samples were not different.

## Discussion

In this study, we examined the growth of *B. thetaiotaomicron* in the presence of Fage plain total 0% Greek yogurt (containing live and active cultures) and in the presence of re-pasteurized Fage plain total 0% Greek yogurt (without live cultures). Based on ANOVA analysis, the numbers of *B. thetaiotaomicron* were not reduced in the presence of Fage plain total 0% Greek yogurt, or in the presence sterile Fage plain total 0% Greek yogurt. Our findings fail to reject the null hypothesis that there is no statistically significant difference in bacterial growth in the presence or absence of yogurt with live cultures. These results are important because they may be used to inform people about the importance of nutrition and its influence on the gut microbiome. With regard to the growing obesity epidemic, this information could aide in informing people that consuming yogurt that contains live and active cultures should not negatively affect the numbers of beneficial *Bacteroides* species in their gut. Furthermore, consuming foods that contain both lactose and casein, which are milk components found within yogurt, also does not appear to affect the growth of *B. thetaiotaomicron.*

The small sample size was a major limitation of this study. Future testing should include much larger sample sizes, as well as testing on a wider range of gut microflora, as *B. thetaiotaomicron* alone is not representative of the vast diversity of microorganisms found within the human gut. The data also had a large variance. The large sample variance can most likely be attributed to two things. First, the McFarland standard is based on visual inspection, and therefore is inherently subjective in its turbidity interpretation (9), and second, the large sample variance could also be attributed to the use of a spot-titer plate for performing colony counts. In terms of the McFarland standard, although the organism was initially diluted to the same McFarland standard in the tubes of thioglycolate

| | ***B. thetaiotaomicron*** | ***B. thetaiotaomicron* with yogurt** | ***B. thetaiotaomicron* with sterile yogurt** |
|---|---|---|---|
| Mean CFU/mL ±SD | 2.35E+07 ± 3.52E+07 | 2.98E+07 ± 4.44E+07 | 3.72E+07 ± 2.94E+07 |
| Sample Variance | 1.23801E+15 | 1.97567E+15 | 8.66763E+14 |

**Table 1.** Data of *B. thetaiotaomicron* grown alone, in the presence of Fage plain total 0% Greek yogurt containing live and active cultures, and in the presence of sterile Fage plain total 0% Greek yogurt. The data show the mean CFUs/mL of bacterial growth of *B. thetaiotaomicron* in a 0.5 McFarland standard after 24 hours of growth, along with the sample variance and SD.

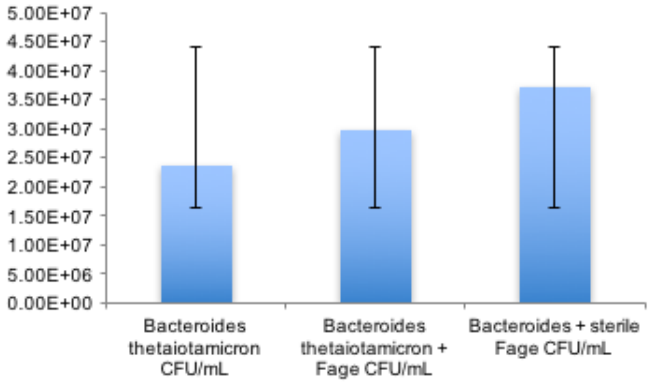


**Figure 1. Mean CFUs/mL of *Bacteroides thetaiotaomicron*.** This image shows the mean CFUs/mL of *B. thetaiotaomicron* growing alone in Thioglycolate broth, in the presence of Fage™ plain total Greek yogurt containing live and active cultures, and in the presence of repasteurized Fage™ plain total Greek yogurt. The error bar shows ±2 SD units.


**Figure 2. Spot titer plates.** This image shows the spot titer plates created from *B. thetaiotaomicron* on reduced BBE agar. From left to right shows *B. thetaiotaomicron* grown with sterilized Fage plain total 0% Greek yogurt, *B. thetaiotaomicron* grown with Fage plain total 0% Greek yogurt, and *B. thetaiotaomicron* grown alone.

broth, small deviations in turbidity could result in different growth densities of the microbe. A way to correct for this would be to calculate the bacterial density of the cultures using optical density readings post-incubation (10). This would allow us to normalize the back-calculations from the number of colonies counted on the spot-titer plates. A method for correcting the spot-titer counts would be to use an entire BBE plate and 1 mL of each dilution tube for the colony counts. This would allow for better separation of the bacteria on the plates and give more accuracy in the enumeration of colonies.

Despite the limitations of this study, the results show that *B. thetaiotaomicron* growth numbers are unaffected when grown in the presence of Fage plain total 0% Greek yogurt containing live and active bacterial cultures. This information can be used to provide guidelines for individuals: consuming yogurts containing live and active bacterial cultures should not decrease the numbers of beneficial Bacteroidete species, such as *B. thetaiotaomicron*, in the gut. Furthermore, the methods of this study could also be used to find ways to increase the numbers of Bacteroidete organisms in the gut, either through the use of more inclusive probiotics supplements or foods, or through prebiotics that can be used to stimulate Bacteroidete growth.

## Materials and Methods

In order to estimate how many CFUs/mL were present in a culture started at a 0.5 McFarland standard that has been grown for 24 hours at 35°C, nine thioglycolate broth solutions (Fluid Thioglycolate Medium Remel – Fisher Science) were inoculated with *B. thetaiotaomicron* to a 0.5 McFarland standard. Next, three of these broths were inoculated with 1 mL of Fage plain total 0% Greek yogurt with live and active bacterial cultures, and three other broths were inoculated with 1 mL of re-pasteurized Fage plain total 0% Greek yogurt. Re-pasteurization of the yogurt was achieved by placing Fage plain total 0% Greek yogurt in an Erlenmeyer flask with a stopper. Each flask was then placed in a 63°C water bath for 30 minutes prior to being used in the study. This method was chosen over membrane filtration because the thickness of the yogurt was too great to be pulled through a vacuum-pressure membrane filter. Additionally, previous studies exploring water bath pasteurization have been shown to be successful in killing microbial organisms in the substance being pasteurized (11). Furthermore, milk and milk products can be pasteurized at a temperature of 63°C for 30 minutes (12). Next, the 9 tubes were incubated at 35°C for 24 hours. After 24 hours of incubation, the thioglycolate broths containing the *B. thetaiotaomicron* alone or with the different yogurt cultures were serially diluted in sterile 0.85% saline to a $10^8$ dilution. Saline was chosen because it is sterile, readily available, and the salt concentration was not presumed to adversely affect the growth of the microbe. From these dilution tubes, spot titers were performed on reduced BBL Bacteroides Bile Esculin agar, a selective agar for growing *Bacteroides* species (Fisher Science) (**Figure 2**). A spot titer was chosen as a way to reduce the number of agar plates used in this experiment, and this technique has been shown to be equally effective as both spread-plate and pour-plate procedures in achieving viable colony counts (4). The BBE plates were incubated anaerobically for 48 hours in a Brewer jar along with a BD GasPak EZ Gas generating system sachet (Fisher Science). The anaerobic system was placed directly into the 35°C incubator. After the plates were incubated for 48 hours, the CFUs/mL were calculated. The spot titer dilution

plate containing 25–250 colonies was chosen, as this numerical range is considered easily countable, and the colony count obtained was divided by 0.01, to account for pipetting 10 µL for the spot titre, and then multiplied by the dilution factor producing 25–250 colonies. This provided an estimate of the CFUs/mL.

## References


1. Armougom F, Henry M, Vialettes B, Raccah D, and Raoult D. 2009. Monitoring Bacterial Community of Human Gut Microbiota Reveals an Increase in *Lactobacillus* in Obese Patients and *Methanogens* in Anorexic Patients. *PLoS ONE* 4(9): e7125. From: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2742902/
2. Benson AK, Kelly SA, Legge R, Ma F, Low SJ, Kim J, Zhang M, Oh PL, Nehrenberg D, Hua K, Kachman SD, Moriyama EN, Walter J, Peterson DA, Pomp D, and Mackay TFC. 2010. Individuality in gut microbiota composition is a complex polygenic trait shaped by multiple environmental and host genetic factors. *Proc Natl Acad Sci* **107** (44):18933-18938.
3. Clemente JC, Ursell LK, Parfrey LW, Knight R. 2012. The Impact of the Gut Microbiota on Human Health: An Integrative View. *Cell* **148** (6):1258–1270. From: http://ac.els-cdn.com/S0092867412001043/1-s2.0-S0092867412001043-main.pdf?_tid=1ed7be78-3c5b-11e4-811b-00000aab0f26&acdnat=1410732458_bab34d9459918e87f7ab60f30bd937b1
4. Gaudy AF, Abu-Niaaj, F, and Gaudy ET. 1962. Statistical Study of the Spot-Plate Technique for Viable-Cell Counts. *Appl. Microbiol* **11**: 305-309.
5. Larsen N, Vogensen FK, Gøbel RJ, Michaelsen KF, Forssten SD, Lahtinen SJ, Jakobsen M. 2013. Effect of Lactobacillus salivarius Ls-33 on fecal microbiota in obese adolescents. *Clin Nutr* **32** (6):935-40
6. Million M, Maraninchi M, Henry M, Armougom F, Richet H, Carrieri P, Valero R, Raccah D, Vialettes B, and Raoult D. 2012. Obesity-associated gut microbiota is enriched in *Lactobacillus reuteri* and depleted in *Bifidobacterium animalis* and *Methanobrevibacter smithii. International Journal of Obesity* **36**: 817–825.
7. Sekirov I, Russell SL, Antunes LCM, and Finlay BB. 2010. Gut Microbiota in Health and Disease. *Physiol Rev*. **90**: 859–904; doi:10.1152/physrev.00045.2009.
8. Zaiontz C. No date. Real Statistics Data Analysis Resource Pack for Excel. From: http://www.real-statistics.com/free-download/real-statistics-resource-pack/
9. Sutton, S. 2006. Measurement of Cell Concentration in Suspension by Optical Density. [Internet]. From: http://www.microbiol.org/resources/monographswhite-papers/measurement-of-cell-concentration-in-suspension-by-optical-density/
10. Myers JA, Curtis BS, and Curtis WR. 2013. Improving accuracy of cell and chromophore concentration measurements using optical density. *BMC Biophysics* **6** (4). doi.org/10.1186/2046-1682-6-4.
11. Worobo R and Padilla-Zakour O. 2016. The development of pasteurization methods for premium fermented apple cider without adding preservatives. *Vivo Research and Expertise Across Cornell*. From: http://vivo.cornell.edu/display/individual16419
12. MyHealth.Alberta.ca. 2015. Food Safety How to Pasteurize Milk at Home. [Online]. 2016 October 25. From: https://myhealth.alberta.ca/Alberta/Pages/how-to-pasteurize-milk.aspx