TYPICAL COLLEGE STUDENT DIET FOOD DERIVED MICROORGANISMS AND THEIR RELATION TO THE HUMAN GASTROINTESTINAL MICROFLORA

A Senior Scholar Thesis

by

ALEXANDRIA JOANN HASELHORST

Submitted to the Office of Undergraduate Research
Texas A&M University
in partial fulfillment of the requirements for the designation as

UNDERGRADUATE RESEARCH SCHOLAR

April 2010

Major: Nutritional Sciences

TYPICAL COLLEGE STUDENT DIET FOOD DERIVED MICROORGANISMS AND THEIR RELATION TO THE HUMAN GASTROINTESTINAL MICROFLORA

A Senior Scholar Thesis

by

ALEXANDRIA JOANN HASELHORST

Submitted to the Office of Undergraduate Research
Texas A&M University
in partial fulfillment of the requirements for the designation as

UNDERGRADUATE RESEARCH SCHOLAR

Approved by:

Research Advisor: Dr. Suresh Pillai
Associate Dean for Undergraduate Research: Robert C. Webb

April 2010

Major: Nutritional Sciences

ABSTRACT

Typical College Student Diet Food Derived Microorganisms and Their Relation to the Human Gastrointestinal Microflora. (April 2010)

Alexandria Joann Haselhorst
Department of Nutrition
Texas A&M University

Research Advisor: Dr. Suresh Pillai
Department of Poultry Science & Nutrition and Food Science

Current research in the field of obesity has shown that obese people generally have a higher percentage of Firmicutes compared to Bacteroidetes in their gastrointestinal tracts than lean people. The goal of this study was to assess whether or not different foods contain unique bacterial profiles and if there is a correlation between foods and the types of bacteria that may be present. This study was based on diets that are typically consumed by college students. Six meals, two breakfasts, two lunches, and two dinners were created based off a class project that detailed the diet of Texas A&M University students. These meals consisted of a banana, cereal, and orange juice; a bagel, breakfast burrito and coffee; a turkey sub sandwich, chips and a soda; macaroni and cheese, carrots and water; pepperoni pizza, salad and water; and finally a cheeseburger, fries and a soda. All the foods were purchased either on campus dining halls, or stores and fast food restaurants in College Station, Texas. Portions of the food samples were plated on Tryptic Soy Agar media and Brucella Blood Agar to determine the aerobic and

anaerobic bacterial loads respectively. The total microbioial communities were extracted from defined amounts of the different food samples and the 16s rRNA genetic diversity were analyzed pyrosequencing. It was found that each food does have a unique bacterial profile. There was, however, no correlation between the nutritional content of the foods and presence of specific bacterial groups. It was determined that on an average, a college student will consume via foods a minimum of about $6.38x10^{10}$ bacterial sequences of bacteria each year, and about $2.55x10^{11}$ sequences of bacteria during an average 4-year college career. The data implies that foods contribute not only nutrients to the human body but can also be a major source for the introduction of microbial populations into the gastrointestinal tract. Since alterations in the gastrointestinal populations do alter host-microbiota interactions which in turn affect metabolic syndromes, this study illustrates one pathway through which microbial populations are adding to the diversity within the gastrointestinal system.

DEDICATION

I would like to dedicate this thesis to my parents who have always believed I could accomplish anything I set my mind to and to my brother Erik for encouraging me to do so.

ACKNOWLEDGMENTS

I would like to thank Dr. Suresh Pillai most of all for all his time and encouragement over this past two years. Also, I would like to thank Dr. Palmy Jesudhasan, Katherine Grace McElhany and the rest of the lab for all their help and resources. I would like to thank Dr. Scot Dowd for performing pyrosequencing for this project. Finally, I would like to thank the Office of Undergraduate Research for providing students the opportunity of undergraduate research and allowing me to take part in the program.

NOMENCLATURE

BBA	Brucella Blood Agar
CFU	Colony Forming Unit
PCR	Polymerase Chain Reaction
TSA	Tryptic Soy Agar

TABLE OF CONTENTS

LIST OF FIGURES

FIGURE Page

LIST OF TABLES

CHAPTER I

INTRODUCTION

College students do not always eat a healthy diet; if they did then the "freshman fifteen" would not be such an issue. What may be surprising is that it may not only be the types of food they are consuming that are making them pack on the pounds but, the bacteria contained on these foods and ultimately influencing their gut microflora. Recently there has been a lot of research on the relationship between gut bacteria and obesity. A major topic of discussion in this field of research is the composition of human gut microflora in relation to those who are obese. The human intestinal microbiota contains trillions of microorganisms (7), which is dominated 90-99% by two bacterial divisions, Bacteroidetes and Firmicutes (7, 8). These two groups of beneficial bacteria produce enzymes to help humans break down otherwise indigestible foods (13) such as digesting complex carbohydrates (19). These enzymes are not present in the absence of the microbiota. This allows humans to absorb more energy from the foods they eat and can result in obesity if there is an excessive positive energy balance (1).

College students are not the only ones having trouble with extra-unwanted weight. Obesity is an epidemic in this country and is one of the leading causes of preventable death worldwide. For years it has been thought that simply taking in more calories, while

---

This thesis follows the style of *Applied and Environmental Microbiology*.

expending less, causes obesity. While this is a major contributing factor, it has been recently proposed that the diversity of a person's gut microbiota can predispose them to obesity. Obese people tend to have a decreased microbial diversity with 50% fewer Bacteroidetes and more Firmicutes than a lean person's gut microbiota (1, 2, 8, 13, 19). This means that a lean person and an obese person can consume the same foods, but the obese person will theoretically gain more weight. This is due to the fact that their gut microbiota is more "efficient" than that of the lean person's, which causes them to absorb more calories from their food. Gaining more weight continues to pre-dispose them to a less diverse microbiota with the more "efficient" bacteria. This predisposition can be reversed with weight loss. It has been shown that previously obese mice that have lost weight on a low calorie diet, have increased their levels of Bacteroidetes and decreased their levels of Firmicutes (13).

Ley *et al*. in 2005 were the first to show the correlation of levels of Bacteroidetes and Firmicutes to obesity in mice. In their experiments they analyzed the distal intestineal microbiota of genetically obese (ob/ob) mice, lean mice (ob/+) and their mothers (ob/+) (12). The mice were fed the same polysaccharide diet. They found a relationship with genetic predisposition to colonization of certain gut microbiota in the same experiment (12). In their studies, they found that regardless of the mice being (ob/ob) genotype, the mice shared a close gut microbiota community with their mother and siblings compared to mice from other litters. This means that microbiota colonization can be inherited and can contribute to predisposition to obesity.

Despite the unpleasant stigma attached to bacteria primarily due to their ability to cause infectious diseases and now to predispose people to obesity, bacteria can have many beneficial impacts on the human body as well. Probiotics have been making recent headway as being beneficial to controlling mechanisms in the body and preventing disease. A probiotic is a viable microbial dietary supplement that beneficially affects the host through the intestinal tract (11). The most common probiotics are *Lactobacillus* and *Bifidobacterium*, which work to control and eliminate pathogenic bacteria residing in the gut. These bacteria achieve this in a number of ways including; out-competing other bacteria for receptors along the intestinal epithelium, releasing antimicrobial compounds that lower the pH in the gut environment, and competing for nutrients in the gastrointestinal tract (11). They have also been shown to increase immunity by promoting nonspecific stimulation of the host immune system. Probiotic bacteria have shown to be useful against allergies, cancer, AIDS, and respiratory and urinary tract infections (11). There are more recent claims that probiotics may also reduce risks of osteoporosis, obesity, and type 2 diabetes but direct evidence is still not yet concrete (11). These probiotics are presently sold commercially in fermented dairy products such as yogurt. Dannon's Activia™ (which contain Bifidus regularis®) and Yakult® (which contains *Lactobacillus casei)* are examples of such product. There are reports that in the future, these probiotics may be found in fermented vegetables and meat (11).

Prebiotics can be defined as non-digestible food that beneficially affects the host by selectively stimulating the growth and/or activity of bacteria in the colon (11). Prebiotics also influence beneficial bacteria in the body. Certain foods like onions,

garlic, leeks, beans, peas, artichokes and some cereals, which are all high fiber foods, encourage beneficial bacteria to take up residence in the human colon. Due to the high fiber in these foods, prebiotics have been shown to be protective against weight gain therefore lowering risk of obesity and diabetes. It is hypothesized that probiotics and prebiotics can work alone or together to help improve the survival of microbiota in the gastrointestinal tract thereby improving one's well being. Prebiotics also have some function in preventing osteoporosis by helping with calcium absorption and retention in the body.

Since the gut contains trillions of microbes, one cannot simply count them all to determine the composition of the microbial diversity. In fact, only ~1% of bacteria can be isolated and independently cultured with current methods (20). Instead, a fairly new field of science called metagenomics has developed to understand the genetic potential or genetic signature of different environments. Metagenomics uses high throughput sequencing of the entire microbial population to assess which types of microbes are present. This method relies on sophisticated sequencing technologies and bioinformatic approaches. The basic approach involves the targeting the 16S subunit of ribosomal RNA to obtain the microbial genetic signature (20). Metagenomics was used by Ley *et al* and in other experiments analyzing the gut microbes of mice and humans. New bioinformatic software is also beginning to surface that assess not only the taxonomic composition of a microbial community, but their functional capacity as well (20). Metagenomics has been used in a number of recent studies to highlight the microbial diversity occurring within different parts of the human body (1, 2, 8, 12, 13, 19,)

Recently researchers have been comparing breast-fed infants to formula-fed infants to understand which infants have a higher nutritional status. What they have found was that the foods we eat when we are very young affect not only the nutrition we receive in our body, but also the types of bacteria that ultimately reside in us. When babies are born their gastrointestinal tracts are sterile and they slowly build up their microbial diversity as they age and eat. When infants are breast-fed, they receive different antibodies, immune factors and bacteria through their mother's milk (16). This helps them build up their immune system as well as their gut microbiota. Since their mothers eat different types of food each day the breast milk will also reflect these changes (16). Infants who receive formula derive no antibodies or immune factors in their milk (16). The formula is pasteurized or sterile, which means no or relatively very few bacterial cells. This makes it harder for formula fed infants to colonize their gut microbiota. Research indicates that breastfed individuals have better developed immune systems than formula fed individuals and one can see why (9). Since we know the foods we eat when we are young affect the outcome of which bacteria will colonize in our gastrointestinal tract, the question remains is whether or not food we eat as adults have this same effect.

The objective of this project was to understand the concentration of bacteria (previously linked to obesity) in foods typically eaten by college students. Previous studies have shown that Bacteroidetes and Firmicutes in our bodies have a role to play in energy conversion and ultimately weight gain. The overall objective of these studies was to understand whether the proportion of these bacteria in foods that are commonly consumed as part of a college student's diet could be a cause of obesity. In this project,

six meals (two breakfasts, two lunches and two dinners) were assembled from a collection of foods typically eaten by Texas A&M University students. The meals included cereal with water and a banana, breakfast burrito with a bagel and coffee, a turkey sandwich from a subway shop along with carrots and a soda, macaroni and cheese with an apple and water, pepperoni pizza with a salad and water, and finally a ground beef sandwich from a fast food restaurant with fries and a soda.

The overall goal of my research project was to determine the microbial populations present, in number and species, on the foods of a typical college student diet. Using this information I calculated an estimate of the total amount of bacterial sequences consumed annually. My underlying hypothesis was that different foods will harbor unique microbial diversity profiles since each food is a unique environment for the different microbes. In addition, I hypothesized that there will be a relation to the amount of fat, especially Trans fat in the food and those microbial populations that are known to be associated with obesity. The underlying hypothesis of this project is that by understanding the microbial communities residing in specific foods it would be possible to formulate diets that could potentially reduce the chances of obesity.

CHAPTER II

METHODS

Food samples and sample processing

Food samples

All food samples used in this study were purchased at food outlets both on the Texas A&M University's campus and throughout College Station, Texas. The samples were purchased and handled normally as if it were meant for consumption by any student on campus. This was to ensure that the study simulated a real-life scenario and the results reflected a real representation of bacteria on the food consumed by students.

Transportation of food products

The food samples were placed immediately into coolers after purchase and then transported to the lab. The samples were then removed from the coolers and placed in a biological safety cabinet for sample processing. This was to ensure that extraneous microbial populations did not contaminate the samples.

Sample processing

Each individual food samples was homogenized in a Seward Stomacher 400 (Brinkman, Westbury, N.Y.) before plating and DNA extraction. For aerobic and anaerobic plating, homogenization ensured that the sample was evenly mixed and that when samples were pipetted onto plates, the results were truly representative of the actual microbial load within the sample.

Homogenization was completed by placing 50 g of food product into a sample bag with a filter insert (VWR, West Chester, Penn.). Some products, such as the drinks, were shaken prior to being weighed to ensure accurate collection of any bacteria present. The filter bag was filled with 450 mL of sterile Butterfield's phosphate buffer when solid samples were being processed. The stomacher filter bag was then placed into a Seward Stomacher 400 (Brinkmann, Westbury, N.Y.) and processed for two minutes on the "high" setting. Liquid food samples were not stomached.

Aerobic and anaerobic plate counts

Aerobic plate counts

After homogenization, 0.1mL of sample was removed from the filtered side of each bag and placed into sterile Eppendorf tubes containing 0.9 mL of Butterfield's dilution buffer in order to create a 10-fold serial dilution. The samples were serially diluted up to a dilution of 10^{-7}. The samples were vortexed between each transfer to make sure that the samples were evenly mixed and the serial dilutions were accurately performed. Aliquots of the dilutions were plated onto Tryptic Soy Agar (Mo Bio Labs, Carlsbad, Calif). The dilutions were plated serially up to a dilution of 10^{-7} of single plating. These dilutions were used for all samples to determine the bacterial loads in each sample. Following plating, the plates were incubated for four days at 27°C in order to allow any bacteria present in the foods time to grow. The plates were removed after incubation and the colonies grown on each plate were counted.

Anaerobic plate counts

The procedures for anaerobic plate counts were almost identical to the aerobic plate counts. The differences in procedure came after the stomacher homogenization of the samples. After homogenization, the food samples were immediately transferred to an anaerobic chamber to ensure as little oxygen as possible came in contact with the samples. The food samples were plated onto Brucella Blood Agar (Anaerobe systems, Morgan Hill, Calif.) plates in an anaerobic hood. The anaerobic hood creates an oxygen free environment to ensure the best conditions for any anaerobic bacteria contained in the food to grow. It is much more reliable and truly anaerobic as compared to a typical GasPak EZ Anaerobe Gas Generating Pouch System with Indicator (BD, Franklin Lakes, N. J.) (16). The following dilutions were plated singly in serial dilutions up to 10^{-7}. The plates were incubated in the anaerobic chamber at 27°C for seven days. After the seven days, the colonies growing on the plates were counted and recorded. The plates were checked regularly without removing them from the anaerobic hood but were allowed to stay in the incubator for longer to allow more chance for growth. Anaerobic bacteria typically take longer grow on plates than aerobic bacteria do (3).

Molecular methods

Total microbial community DNA extraction from food products

After homogenization in the stomacher, a 125mL volume of food sample was placed into a sterile centrifuge bottle. The sample was centrifuged at 8,000 x g for 10 minutes. The supernatant was discarded and another 125 mL of homogenized food sample was added

and centrifuged for an additional 10 minutes. The supernatant was again discarded and 50 mL of Butterfield's phosphate buffer was added to centrifuge bottle and then vortexed to resuspend the pellet before being centrifuged for another 10 minutes. The supernatant was discarded again and the pellet was resuspended by 4 mL of Butterfield's. This procedure was altered for the liquid foods , which were already in a liquid state. Instead of being mixed with Butterfield's and homogenized by the stomacher, they were simply added directly to a sterile centrifuge bottle.

DNA was extracted from all food samples using a Maximum Yield Protocol of Ultra Clean Soil DNA Kit (MoBio, Carlsbad, Calif.). There is currently no special DNA kit for extracting DNA from food so a soil DNA kit was used instead to obtain the best results. This protocol can be found in Appendix A. The extracted DNA was analyzed for yield and purity using a NanoDrop spectrophotometer and stored at -20°C for later use.

Pyrosequencing of food sample community DNA

DNA extracted from food samples were sent to Medical Biofilm Research Institute in Lubbock, Texas for analysis of metagenomic content. Each food sample with the highest concentration of extracted DNA nucleic acids and purity shown by the spectrophotometer were selected for pyrosequencing. The results of the pyrosequencing were sent back from the lab and analyzed to determine the microbial diversity of the bacteria present in the food.

CHAPTER III

RESULTS

Culture-based methods

Bacterial loads on food samples were analyzed aerobically on Tryptic Soy Agar as well as anaerobically on Brucella Blood Agar. Results showed that of the 16 foods plated under aerobic conditions 9 had significant bacterial loads in the food: banana, bagel, breakfast burrito, coffee, baby carrots, salad, burger, fries and sandwich (Table 1). Of these 9 foods all showed an anaerobic bacterial load except the bagel. The foods that showed no bacterial load were pepperoni pizza, coke, apple, cereal, macaroni and cheese, orange juice, and water.

The range of anaerobic bacterial loads differed depending on the food. Of the foods examined, only the salad and burger showed anaerobic bacterial loads similar to their aerobic ones. The breakfast burrito, fries and the sandwich all showed anaerobic bacterial loads greater than their aerobic bacterial loads. The banana, coffee, and baby carrots had anaerobic bacterial loads less than their aerobic loads. Finally, the bagel showed no anaerobic bacterial load at all.

TABLE 1. Aerobic and anaerobic bacterial load on food samples.

Food Sample	*Aerobic Load*	*Anaerobic Load*
Banana	$8x10^2$ CFU/g	3 CFU/g
Bagel	$3.9x10^1$ CFU/g	Below detection limit[a]
Breakfast Burrito	$5.5x10^5$ CFU/g	$6.0x10^5$ CFU/g
Coffee	$1.7\ x10^2$ CFU/mL	$3.3x10^1$ CFU/mL
Baby Carrots	$1.2x10^5$ CFU/g	$2.0x10^4$ CFU/g
Salad	$6.8x10^5$ CFU/g	$5.3x10^5$ CFU/g
Burger	$3.5x10^2$ CFU/g	$8.8x10^1$ CFU/g
Fries	1.0 CFU/g	$3.0x10^2$ CFU/g
Sandwich	$1.1x10^6$ CFU/g	$2.8x10^6$ CFU/g

[a] No colonies grew on the plate.

Pyrosequencing results

The pyrosequencing data analysis of the food samples were based on the 16S database. The data was analyzed taxonomically at the phylum, genus and species level for each food product. The results will focus mainly on the phylum and genus levels starting with each meal as a whole and then focusing on each food independently. Nine different bacterial phyla were present on the foods namely Firmicutes, Nitrospirae, Fusobacteria, Actinobacteria, Bacteroidetes, Proteobacteria, Tenericutes, Cyanobacteria, and Deinococcus-Thermus. The results will focus on the phylum's Firmicutes, Bacteroidetes, and Proteobacteria as they were found in the greatest amounts in the foods. The only phylum that was present in greater amounts was Cyanobacteria. However, they were in such high amounts that other phyla were not represented at all in the graphs. For, this reason Cyanobacteria will be disregarded in the results. A complete list of the all the bacterial genera found in the foods can be found in Appendix C. These results reflect the amount of sequences in 1µl of DNA analyzed from each food. The amount of DNA that would be in a serving size of each food was calculated and will also be discussed. These calculations can be found in Appendix D.

Breakfast one

Breakfast one consisted of cereal, a banana and water. Pyrosequencing analysis of the foods in Breakfast one revealed a total of 25,554 sequences of the nine phyla of which 1112 sequences coming from Firmicutes, Bacteroidetes and Proteobacteria. The genera present in these phyla were also determined from the 16S database. Breakfast one limited to these phyla comprised of 92.8% Firmicutes, 6.9% Proteobacteria and 0.003%

Bacteroidetes (Fig1). This meal as a whole contains $1.42x10^8$ sequences of bacteria in 408.8 g of food.

Cereal

The cereal analyzed was Honey Bunches of Oats with Almonds. Pyrosequencing analysis of cereal revealed a total of 9,474 sequences, 90 which were further analyzed for identification of the genera present (Table 2). Of the sequences rejected from the analysis 9,382 came from Cyanobacteria. A phylogenetic analysis limited to only Firmicutes, Proteobacteria and Bacteroidetes show that cereal consisted of 87.78% Firmicutes, 10% Proteobacteria and 2.22% Bacteroidetes (Fig.2). A typical serving size of cereal is one cup or 32g which would contain $1.50x10^7$ sequences of bacteria.

Banana

The banana analyzed was a Yellow Plantain banana. Pyrosequencing analysis of the banana showed a total of 14,579 sequences, 9 of which were further analyzed for identification of genera present (Table 3). Of the sequences rejected 14,569 came from Cyanobacteria. A phylogenetic analysis limited to only Firmicutes and Proteobacteria shows that cereal consisted of 44.44% Firmicutes and 55.56% Proteobacteria (Fig. 3). No Bacteroidetes were found in the banana. A cup of bananas is approximately 150g and would contain $1.09x10^8$ sequences of bacteria.

Water

Water analyzed was tap water from College Station, TX. Pyrosequencing analysis of

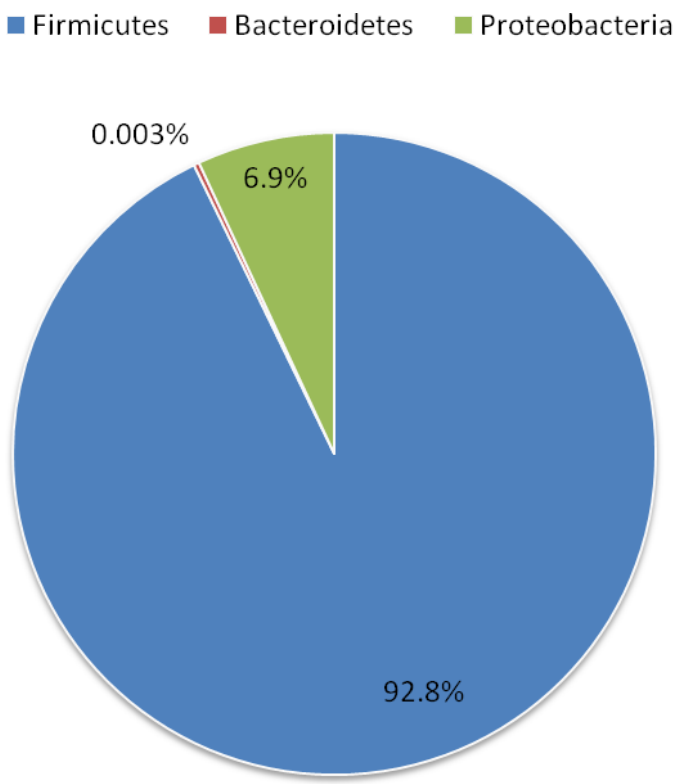

FIGURE 1. Phylogenetic analysis of the metagenomic content of breakfast one determined by pyrosequencing

TABLE 2. Results of pyrosequencing analysis of cereal.

Genus	***Phylum***	***Number of Sequences***
Anaerococcus spp.	*Firmicutes*	1
Bacteroides spp.	*Bacteroidetes*	1
Clostridium spp.	*Firmicutes*	1
Geobacillus spp.	*Firmicutes*	5
Lactobacillus spp.	*Firmicutes*	1
Lactococcus spp.	*Firmicutes*	1
Lutibacter spp.	*Bacteroidetes*	1
Macrococcus spp.	*Firmicutes*	3
Pannonibacter spp.	*Proteobacteria*	2
Peptostreptococcus spp.	*Firmicutes*	2
Pseudomonas spp.	*Proteobacteria*	4
Streptococcus spp.	*Firmicutes*	65
Sulfurovum spp.	*Proteobacteria*	2
Syntrophorhabdus spp.	*Proteobacteria*	1
Total		**90**

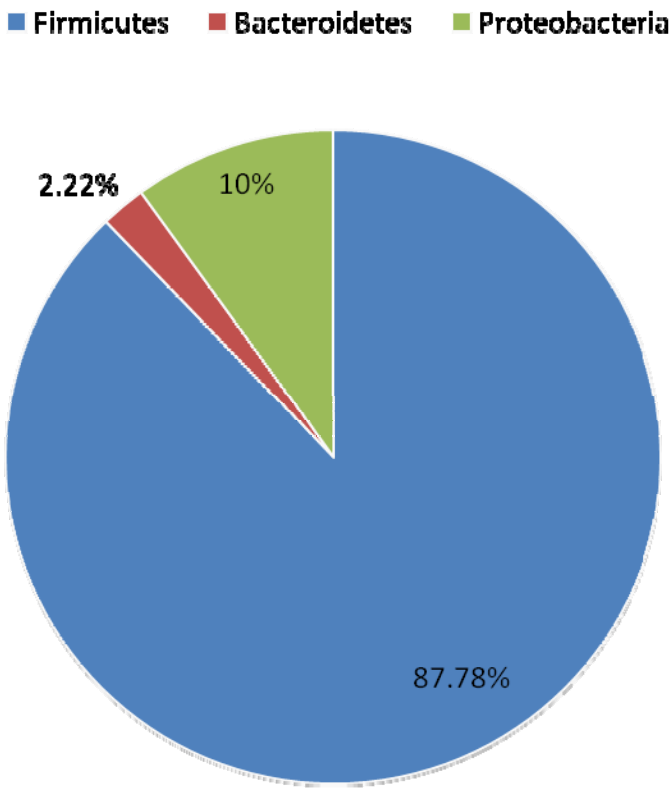

FIGURE 2. Phylogenetic analysis of the metagenomic content of cereal determined by pyrosequencing.

TABLE 3. Results of pyrosequencing analysis of banana.

Genus	***Phylum***	***Number of Sequences***
Dorea spp.	*Firmicutes*	1
Kozakia spp.	*Proteobacteria*	3
Pseudomonas spp.	*Proteobacteria*	1
Staphylococcus spp.	*Firmicutes*	2
Streptococcus spp.	*Firmicutes*	1
Thioalkalivibrio spp.	*Proteobacteria*	1
Total		**9**

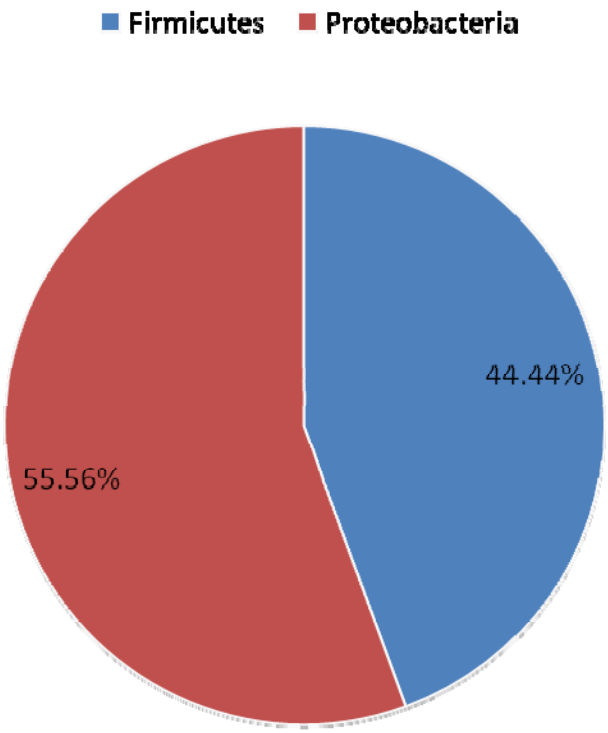

FIGURE 3. Phylogenetic analysis of the metagenomic content of banana determined by pyrosequencing.

water showed a total of 1501 sequences, 1013 which were further analyzed for identification of genera present (Table 4). Of the sequences rejected from final analysis, 487 came from Cyanobacteria. A phylogenetic analysis limited to Firmicutes, Proteobacteria and Bacteroidetes shows that water consisted of 93.68% Firmicutes, 6.22% Proteobacteria and 9.87×10^{-4}% Bacteroidetes (Fig 4). There is roughly 226.8g of water in 8 fluid ounces which would contain 1.70×10^{7} sequences of bacteria.

Breakfast two

Breakfast two consisted of a breakfast burrito, a bagel and coffee with milk. Pyrosequencing analysis of the foods in breakfast two showed a total of 22,303 sequences, 19,832 that were used for further analysis for identification of genera present. Of the sequences rejected from analysis, 2,391 came from Cyanobacteria. A phylogenetic analysis limited to Firmicutes, Proteobacteria and Bacteroidetes showed breakfast two consisted of 96.41% Firmicutes, 3.35% Proteobacteria and 0.002% Bacteroidetes (Fig. 5). If the servings sizes suggested below were consumed then a person would be consuming about 1.96×10^{8} sequences of bacteria in 477.8 g of food.

Breakfast burrito

The breakfast burrito consisted of a flour tortilla, scrambled eggs, cheddar cheese and hash browns. Pyrosequencing analysis of the breakfast burrito showed a total of 13,054 sequences, 13,034 that were used for further analysis for identification of genera present (Table 5). Of the sequences rejected from analysis, 18 came from Cyanobacteria. A phylogenetic analysis limited to Firmicutes and Proteobacteria showed breakfast burrito

TABLE 4. Results of pyrosequencing analysis of water.

Genus	***Phylum***	***Number of Sequences***
Acidovorax spp.	*Proteobacteria*	5
Anaerococcus spp.	*Firmicutes*	7
Anoxybacillus spp.	*Firmicutes*	55
Bacillus spp.	*Firmicutes*	35
Bacteroides spp.	*Bacteroidetes*	1
Clostridium spp.	*Firmicutes*	2
Delftia spp.	*Proteobacteria*	18
Geobacillus spp.	*Firmicutes*	5
Klebsiella spp.	*Proteobacteria*	1
Lactobacillus spp.	*Firmicutes*	14
Lactococcus spp.	*Firmicutes*	491
Leucothrix spp.	*Proteobacteria*	1
Methylobacterium spp.	*Proteobacteria*	6
Paenibacillus spp.	*Firmicutes*	11
Pelomonas spp.	*Proteobacteria*	4
Pseudomonas spp.	*Proteobacteria*	7
Salmonella spp.	*Proteobacteria*	7
Stenotrophomonas spp.	*Proteobacteria*	12
Streptococcus spp.	*Firmicutes*	318
Sulfurovum spp.	*Proteobacteria*	1
Syntrophorhabdus spp.	*Proteobacteria*	1
Thermolithobacter spp.	*Firmicutes*	1
Total		**1003**

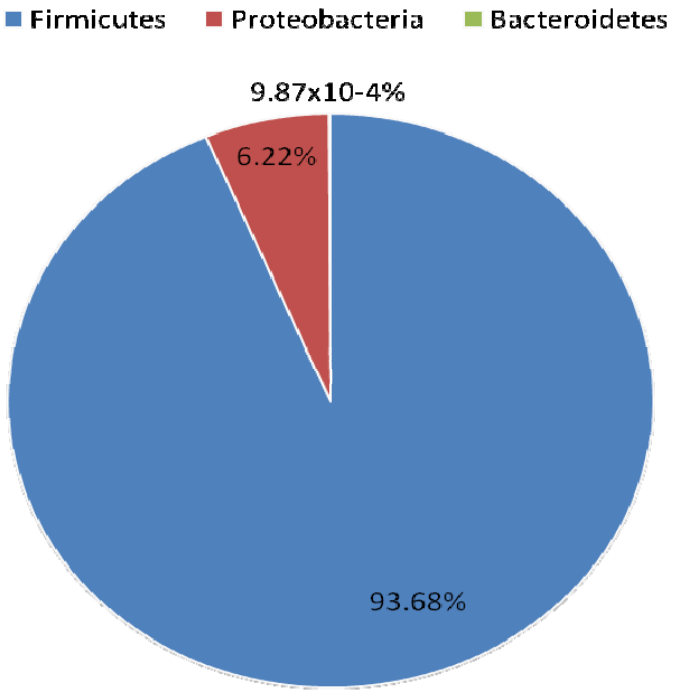

FIGURE 4. Phylogenetic analysis of the metagenomic content of water determined by pyrosequencing.

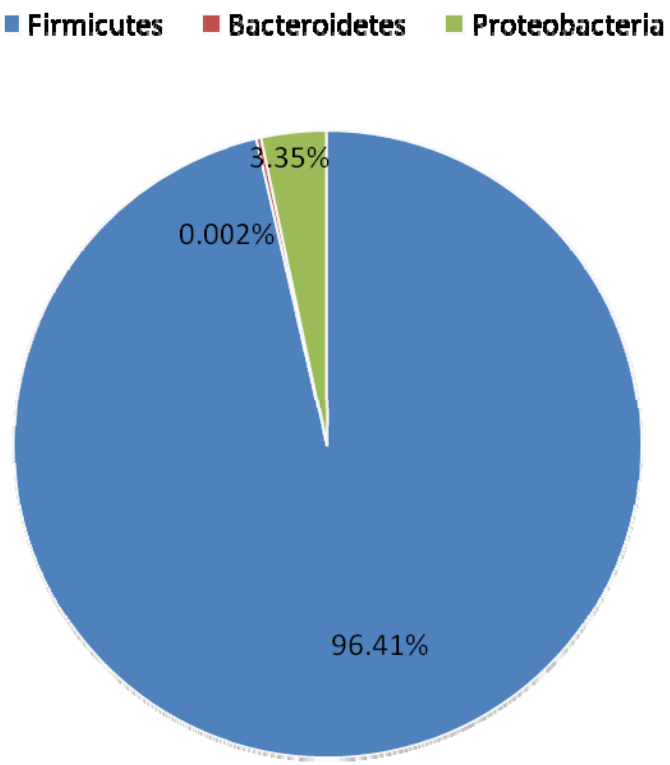

FIGURE 5. Phylogenetic analysis of the metagenomic content of breakfast two determined by pyrosequencing.

TABLE 5. Results of pyrosequencing analysis of breakfast burrito.

Genus	*Phylum*	*Number of Sequences*
Anoxybacillus spp.	*Firmicutes*	19
Bacteroides spp.	*Bacteroidetes*	0
Brevibacillus spp.	*Firmicutes*	3
Erythrobacter spp.	*Proteobacteria*	1
Exiguobacterium spp.	*Firmicutes*	1
Halomonas spp.	*Proteobacteria*	1
Lactobacillus spp.	*Firmicutes*	4
Lactococcus spp.	*Firmicutes*	11232
Macrococcus spp.	*Firmicutes*	5
Marinobacter spp.	*Proteobacteria*	3
Prevotella spp.	*Bacteroidetes*	1
Proteus spp.	*Proteobacteria*	1
Pseudomonas spp.	*Proteobacteria*	3
Staphylococcus spp.	*Firmicutes*	1
Stenotrophomonas spp.	*Proteobacteria*	1
Streptococcus spp.	*Firmicutes*	1757
Veillonella spp.	*Firmicutes*	1
Total		**13034**

consisted of 99.92% Firmicutes, 0.08% Proteobacteria (Fig. 6). There was one Bacteroidetes sequence in the breakfast burrito. A serving size of this breakfast burrito is 141g and contains $9.18x10^7$ sequences of bacteria.

Bagel

The bagel was a plain white flour bagel. Pyrosequencing analysis of the bagel showed a total of 2,428 sequences, 165 that were used for further analysis for identification of genera present (Table 6). Of the sequences rejected from analysis, 2,252 came from Cyanobacteria. A phylogenetic analysis limited to Firmicutes, Proteobacteria and Bacteroidetes showed the bagel consisted of 39.39% Firmicutes, 59.39% Proteobacteria and 1.21% Bacteroidetes (Fig. 7). A bagel weights 110g and would contain $1.34x10^7$ sequences of bacteria.

Coffee

The coffee analyzed was 12th Man Brew™ caffeinated coffee with half & half added to the coffee. Pyrosequencing analysis coffee showed a total of 6,821sequences, 6,633 that were used for further analysis for identification of genera present (Table 7). Of the sequences rejected from analysis, 121 came from Cyanobacteria. A phylogenetic analysis limited to Firmicutes, Proteobacteria and Bacteroidetes showed coffee consisted of 90.94% Firmicutes, 8.38% Proteobacteria and 0.68% Bacteroidetes (Fig. 8). In 8 oz of coffee there would be about $7.74x10^7$ sequences of bacteria.

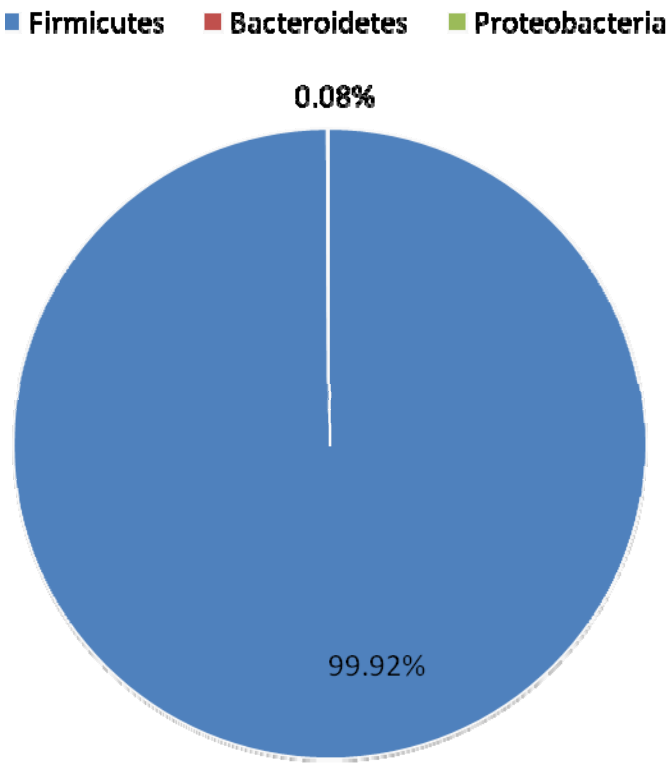

FIGURE 6. Phylogenetic analysis of the metagenomic content of breakfast burrito determined by pyrosequencing.

TABLE 6. Results of pyrosequencing analysis of bagel.

Genus	*Phylum*	*Number of Sequences*
Catonella spp.	*Firmicutes*	4
Delftia spp.	*Proteobacteria*	1
Devosia spp.	*Proteobacteria*	3
Endoriftia spp.	*Proteobacteria*	1
Flavobacterium spp.	*Bacteroidetes*	2
Lactococcus spp.	*Firmicutes*	16
Leuconostoc spp.	*Firmicutes*	23
Paenibacillus spp.	*Firmicutes*	10
Pantoea spp.	*Proteobacteria*	15
Paracoccus spp.	*Proteobacteria*	12
Pectobacterium spp.	*Proteobacteria*	13
Prevotella spp.	*Bacteroidetes*	0
Pseudomonas spp.	*Proteobacteria*	46
Salmonella spp.	*Proteobacteria*	1
Serratia spp.	*Proteobacteria*	3
Staphylococcus spp.	*Firmicutes*	1
Streptococcus spp.	*Firmicutes*	11
Tuberoidobacter spp.	*Proteobacteria*	1
Xylophilus spp.	*Proteobacteria*	2
Total		**165**

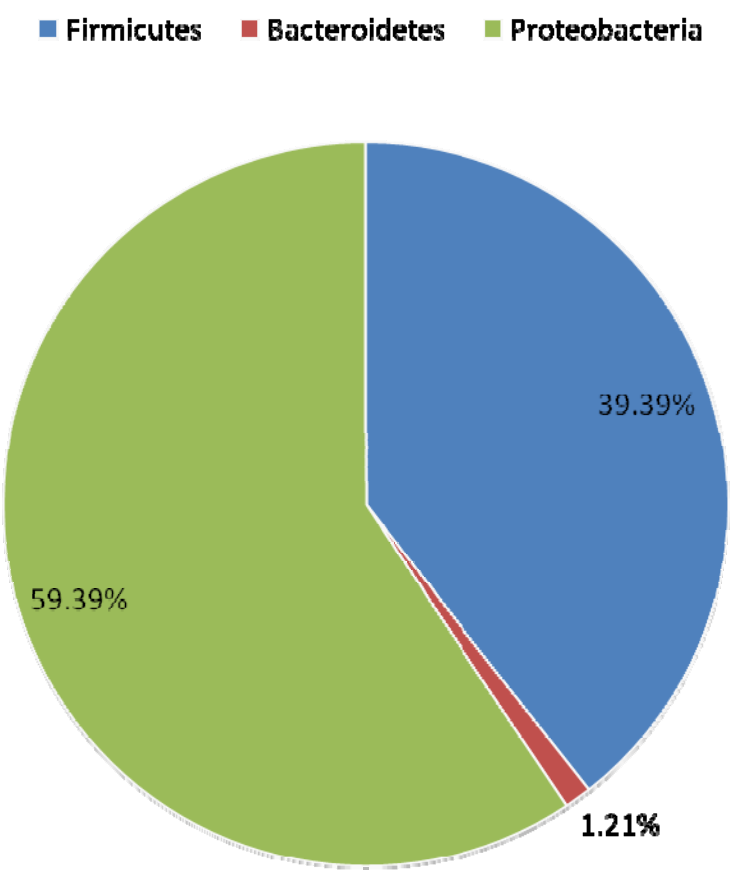

FIGURE 7. Phylogenetic analysis of the metagenomic content of bagel determined by pyrosequencing.

TABLE 7. Results of pyrosequencing analysis of coffee.

Genus	*Phylum*	*Number of Sequences*
Achromobacter spp.	*Proteobacteria*	1
Acinetobacter spp.	*Proteobacteria*	154
Aerococcus spp.	*Firmicutes*	4
Alcaligenes spp.	*Proteobacteria*	2
Alistipes spp.	*Bacteroidetes*	14
Anaerofilum spp.	*Firmicutes*	1
Anaerovorax spp.	*Firmicutes*	22
Anoxybacillus spp.	*Firmicutes*	5514
Atopostipes spp.	*Firmicutes*	3
Bacillus spp.	*Firmicutes*	28
Bacteroides spp.	*Bacteroidetes*	16
Butyrivibrio spp.	*Firmicutes*	1
Chryseobacterium spp.	*Bacteroidetes*	1
Citrobacter spp.	*Proteobacteria*	14
Clostridium spp.	*Firmicutes*	167
Enterobacter spp.	*Proteobacteria*	2
Enterococcus spp.	*Firmicutes*	12
Exiguobacterium spp.	*Firmicutes*	0
Fastidiosipila spp.	*Firmicutes*	1
Flavobacterium spp.	*Bacteroidetes*	12
Geobacillus spp.	*Firmicutes*	5
Gracilibacter spp.	*Firmicutes*	5
Guggenheimella spp.	*Firmicutes*	14
Halomonas spp.	*Proteobacteria*	2
Helcococcus spp.	*Firmicutes*	1
Herbaspirillum spp.	*Proteobacteria*	2
Janthinobacterium spp.	*Proteobacteria*	2
Jeotgalicoccus spp.	*Firmicutes*	9
Lactobacillus spp.	*Firmicutes*	8
Lactococcus spp.	*Firmicutes*	1
Listeria spp.	*Firmicutes*	3
Lysinibacillus spp.	*Firmicutes*	1
Macrococcus spp.	*Firmicutes*	17

TABLE 7. Continued.

Genus	*Phylum*	*Number of Sequences*
Marinimicrobium spp.	*Proteobacteria*	2
Marinobacter spp.	*Proteobacteria*	6
Oceanobacter spp.	*Proteobacteria*	1
Paenibacillus spp.	*Firmicutes*	9
Pantoea spp.	*Proteobacteria*	5
Paracoccus spp.	*Proteobacteria*	11
Pectobacterium spp.	*Proteobacteria*	9
Pelomonas spp.	*Proteobacteria*	26
Petrimonas spp.	*Bacteroidetes*	1
Planococcus spp.	*Firmicutes*	2
Prevotella spp.	*Bacteroidetes*	1
Pseudomonas spp.	*Proteobacteria*	100
Roseburia spp.	*Firmicutes*	6
Roseinatronobacter spp.	*Proteobacteria*	4
Roseomonas spp.	*Proteobacteria*	4
Ruminococcus spp.	*Firmicutes*	3
Sphingomonas spp.	*Proteobacteria*	15
Sphingopyxis spp.	*Proteobacteria*	5
Sporobacter spp.	*Firmicutes*	2
Stenotrophomonas spp.	*Proteobacteria*	189
Streptococcus spp.	*Firmicutes*	164
Trichococcus spp.	*Firmicutes*	11
Turicibacter spp.	*Firmicutes*	18
Total		**6633**

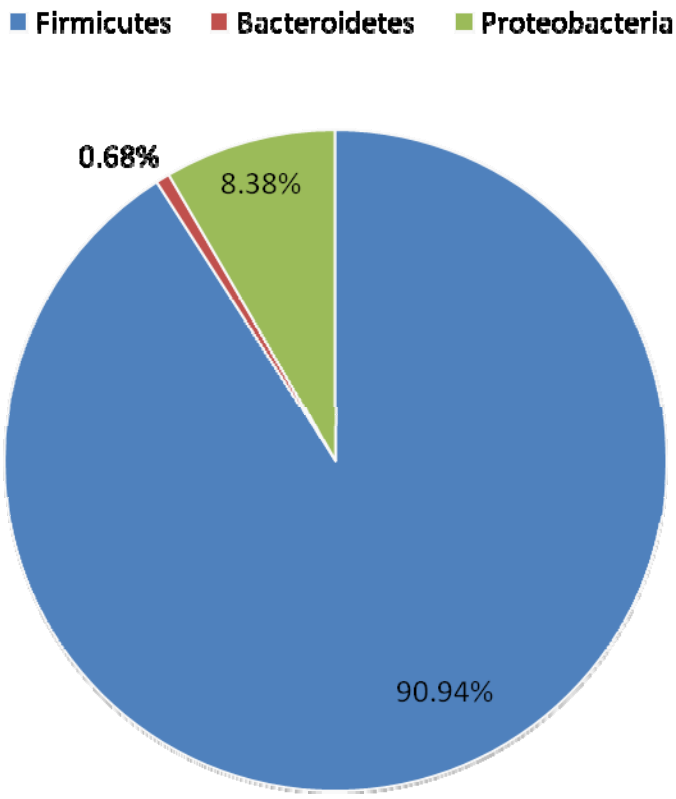

FIGURE 8. Phylogenetic analysis of the metagenomic content of coffee determined by pyrosequencing.

Lunch one

Lunch one consisted of macaroni & cheese, an apple and water. Pyrosequencing analysis of the foods in lunch one showed a total of 5,618 sequences, 4,098 that were used for further analysis for identification of genera present. Of the sequences rejected from analysis, 1,516 came from Cyanobacteria. A phylogenetic analysis limited to Firmicutes, Proteobacteria and Bacteroidetes showed lunch one consisted of 92.90% Firmicutes, 7.08% Proteobacteria and $2.44x10^{-4}$% Bacteroidetes (Fig. 9). This meal as a whole contains $3.47x10^{7}$ sequences of bacteria in 434.8 g of food.

Macaroni & cheese

The macaroni & cheese analyzed was Easy Mac™ macaroni & cheese. Pyrosequencing analysis of macaroni & cheese showed a total of 2,872 sequences, 2,866 that were used for further analysis for identification of genera present (Table 8). Of the sequences rejected from analysis, 3 came from Cyanobacteria. A phylogenetic analysis limited to Firmicutes and Proteobacteria showed macaroni & cheese consisted of 99.69% Firmicutes 0.21% Proteobacteria (Fig. 10). There were no Bacteroidetes found in macaroni & cheese. One cup of macaroni and cheese is 58g and would contain $8.33x10^{6}$ sequences of bacteria.

Apple

The apple analyzed was a Jonagold™ apple. Pyrosequencing analysis of the apple showed a total of 1,245sequences, 219 that were used for further analysis for identification of genera present (Table 9). Of the sequences rejected from analysis, 1,026

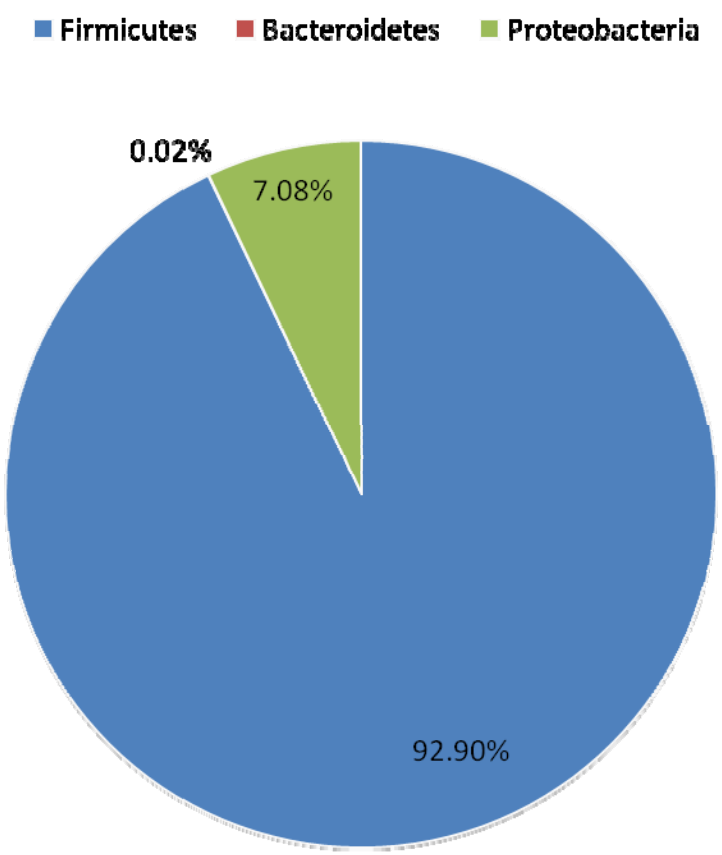

FIGURE 9. Phylogenetic analysis of the metagenomic content of lunch one determined by pyrosequencing.

TABLE 8. Results of pyrosequencing analysis of macaroni & cheese.

Genus	***Phylum***	***Number of Sequences***
Acinetobacter	*Proteobacteria*	3
Anoxybacillus	*Firmicutes*	15
Bacillus	*Firmicutes*	1
Geobacillus	*Firmicutes*	1381
Lactobacillus	*Firmicutes*	21
Lactococcus	*Firmicutes*	81
Leucothrix	*Proteobacteria*	2
Pediococcus	*Firmicutes*	2
Stenotrophomonas	*Proteobacteria*	4
Streptococcus	*Firmicutes*	1355
Turicibacter	*Firmicutes*	1
Total		**2866**

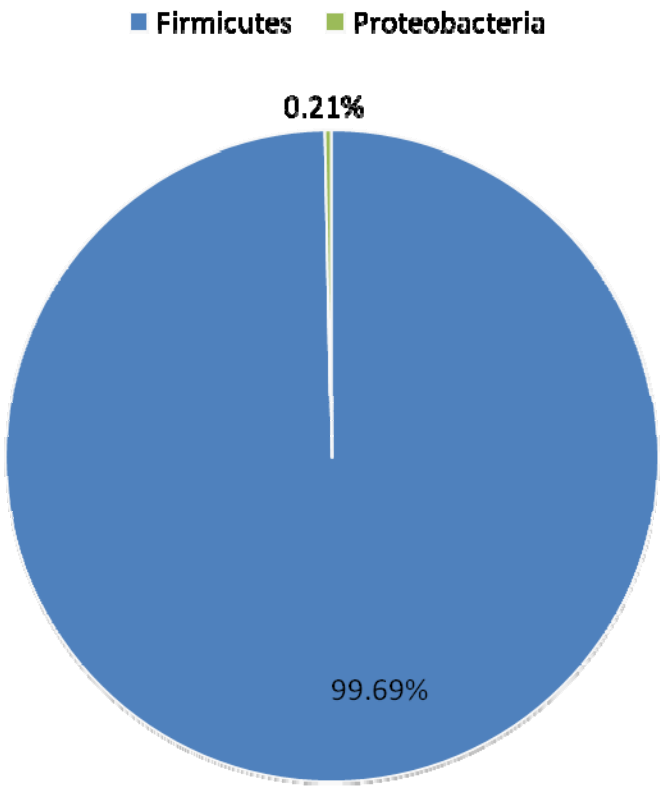

FIGURE 10. Phylogenetic analysis of the metagenomic content of macaroni & cheese determined by pyrosequencing.

TABLE 9. Results of pyrosequencing analysis of apple.

Genus	***Phylum***	***Number of Sequences***
Comamonas	*Proteobacteria*	46
Diaphorobacter	*Proteobacteria*	15
Halochromatium	*Proteobacteria*	1
Pseudomonas	*Proteobacteria*	59
Rubellimicrobium	*Proteobacteria*	97
Sporobacter	*Firmicutes*	1
Total		**219**

came from Cyanobacteria. A phylogenetic analysis limited to Firmicutes and Proteobacteria showed the apple consisted of 0.46% Firmicutes 99.54% Proteobacteria (Fig. 11). No Bacteroidetes sequences were found in the apple. An average apple is 150g and would contain $9.34x10^6$ sequences of bacteria.

Water

Pyrosequencing analysis of water was the same as found in breakfast one.

Lunch two

Lunch Two consisted of a turkey sandwich, carrots and soda. Pyrosequencing analysis of the foods in lunch two showed a total of 17,856 sequences, 9,892 that were used for further analysis for identification of genera present. Of the sequences rejected from analysis, 7,956 came from Cyanobacteria. A phylogenetic analysis limited to Firmicutes, Proteobacteria and Bacteroidetes showed lunch two consisted of 13.5% Firmicutes, 85.01% Proteobacteria and 1.49% Bacteroidetes (Fig. 12). As a whole this meal contains $1.24x10^8$ sequences of bacteria in 540.8g of food.

Turkey sandwich

The turkey sandwich contained wheat bread, light mayonnaise, yellow mustard, turkey, American cheese, tomatoes, lettuce and pickles. Pyrosequencing analysis of the turkey sandwich showed a total of 17,856 sequences, 9,892 that were used for further analysis for identification of genera present (Table 10). Of the sequences rejected from analysis, 7,956 came from Cyanobacteria. A phylogenetic analysis limited to Firmicutes and

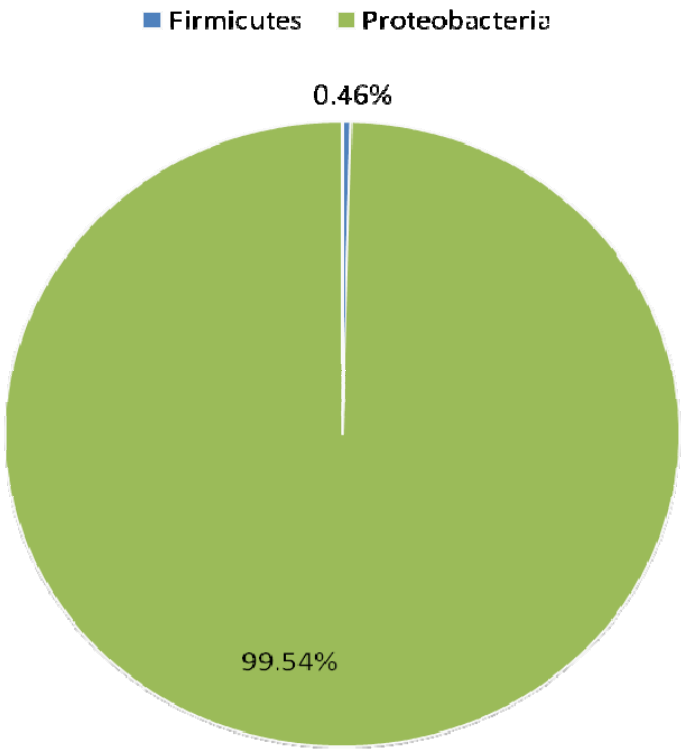

FIGURE 11. Phylogenetic analysis of the metagenomic content of apple determined by pyrosequencing.

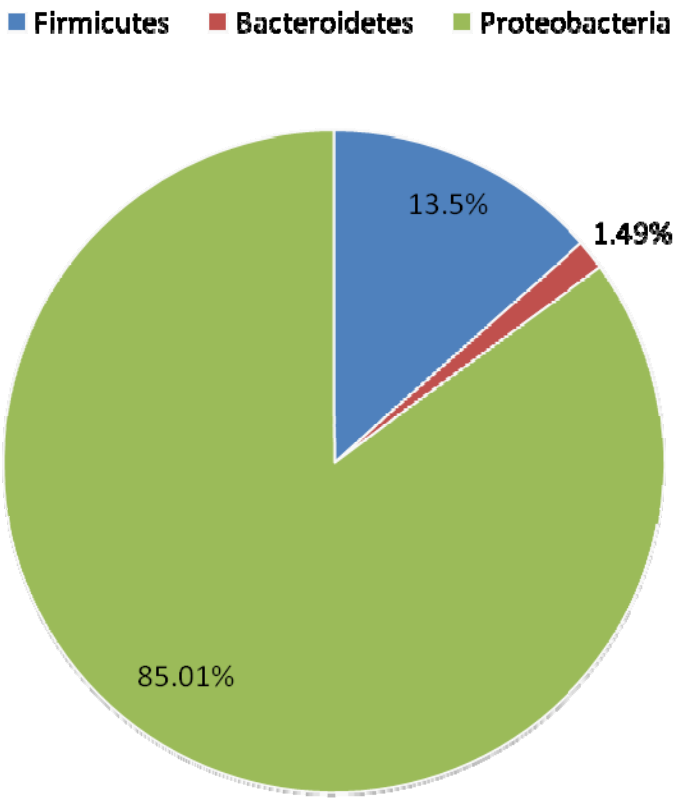

FIGURE 12. Phylogenetic analysis of the metagenomic content of lunch two determined by pyrosequencing

TABLE 10. Results of pyrosequencing analysis of turkey sandwich.

Genus	***Phylum***	***Number of Sequences***
Brochothrix	*Firmicutes*	1
Erwinia	*Proteobacteria*	3
Klebsiella	*Proteobacteria*	2
Lactobacillus	*Firmicutes*	908
Lactococcus	*Firmicutes*	88
Leuconostoc	*Firmicutes*	31
Leucothrix	*Proteobacteria*	1
Marinobacter	*Proteobacteria*	1
Microvirga	*Proteobacteria*	0
Oceanimonas	*Proteobacteria*	1
Pantoea	*Proteobacteria*	3
Parasporobacterium	*Firmicutes*	1
Pectobacterium	*Proteobacteria*	3
Pseudomonas	*Proteobacteria*	6
Serratia	*Proteobacteria*	1
Shewanella	*Proteobacteria*	61
Staphylococcus	*Firmicutes*	2
Streptococcus	*Firmicutes*	292
Vibrio	*Proteobacteria*	5670
Total		**1405**

Proteobacteria showed the turkey sandwich consisted of 18.7% Firmicutes, 81.3% Proteobacteria (Fig. 13). There were no Bacteroidetes found on the turkey sandwich. The weight of this sandwich is about 250g and contains a total of $9.01x10^7$ sequences of bacteria.

Carrots

Pyrosequencing analysis of carrots showed a total of 10,626 sequences, 2,806 that were used for further analysis for identification of genera present (Table 11). Of the sequences rejected from analysis, 7,820 came from Cyanobacteria. A phylogenetic analysis limited to Firmicutes, Proteobacteria and Bacteroidetes showed carrots consisted of 0.2% Firmicutes, 5.25% Proteobacteria and 94.55% Bacteroidetes (Fig. 14). A serving size of ½ cup, 64g, of uncooked carrots has a total of $3.40x10^7$ sequences of bacteria.

Soda

The soda analyzed was Coca-Cola classic coke™. Pyrosequencing analysis of soda showed a total of 19 sequences, 11 that were used for further analysis for identification of genera present (Table 12). Of the sequences rejected from analysis, none came from Cyanobacteria. A phylogenetic analysis limited to Firmicutes and Proteobacteria showed soda consisted of 54.55% Firmicutes, 45.45% Proteobacteria (Fig. 15). There were no Bacteroidetes found in soda. There are a total of $2.15x10^5$ sequences of bacteria in 8oz (226.8g) of coke.

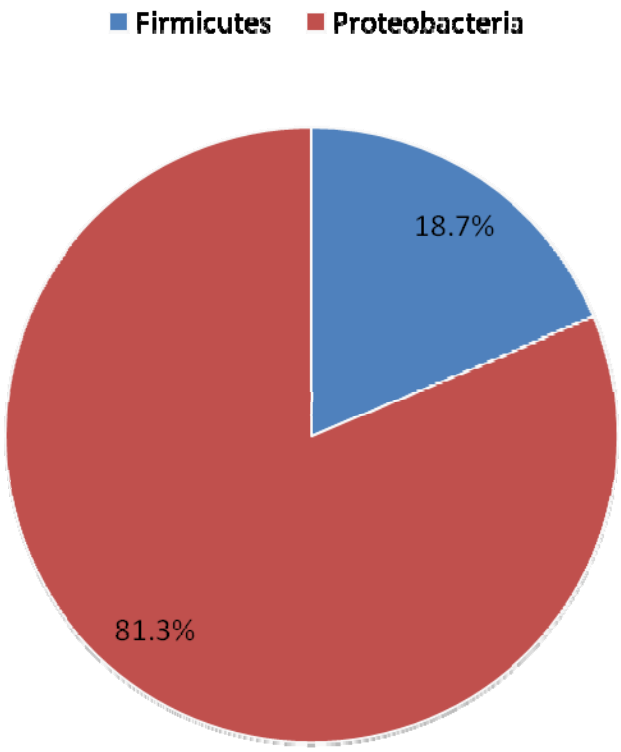

FIGURE 13. Phylogenetic analysis of the metagenomic content of turkey sandwich determined by pyrosequencing.

TABLE 11. Results of pyrosequencing analysis of carrots.

Genus	***Phylum***	***Number of Sequences***
Bacteroides	*Bacteroidetes*	1
Buttiauxella	*Proteobacteria*	1
Enterococcus	*Firmicutes*	2
Erwinia	*Proteobacteria*	5
Faecalibacterium	*Firmicutes*	4
Flavobacterium	*Bacteroidetes*	144
Janthinobacterium	*Proteobacteria*	24
Klebsiella	*Proteobacteria*	5
Leucothrix	*Proteobacteria*	3
Pannonibacter	*Proteobacteria*	4
Pectobacterium	*Proteobacteria*	373
Pedobacter	*Bacteroidetes*	2
Pseudomonas	*Proteobacteria*	2151
Rahnella	*Proteobacteria*	25
Raoultella	*Proteobacteria*	2
Serratia	*Proteobacteria*	30
Sulfurovum	*Proteobacteria*	1
Syntrophorhabdus	*Proteobacteria*	2
Thiothrix	*Proteobacteria*	1
Yersinia	*Proteobacteria*	26
Total		**2806**

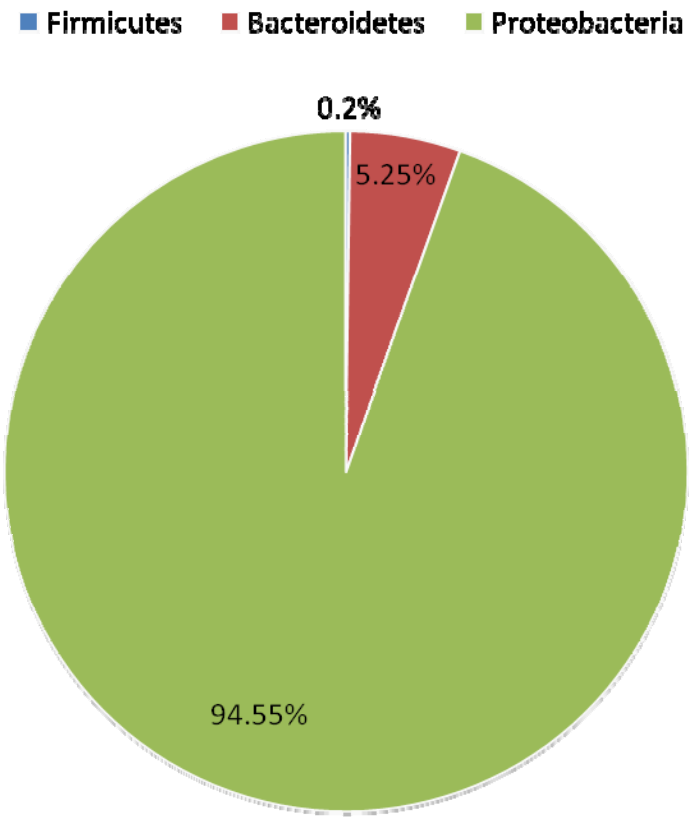

FIGURE 14. Phylogenetic analysis of the metagenomic content of carrots determined by pyrosequencing.

TABLE 12. Results of pyrosequencing analysis of soda.

Genus	***Phylum***	***Number of Sequences***
Staphylococcus	*Firmicutes*	6
Microvirga	*Proteobacteria*	1
Pseudomonas	*Proteobacteria*	2
Pelomonas	*Proteobacteria*	2
Total		9

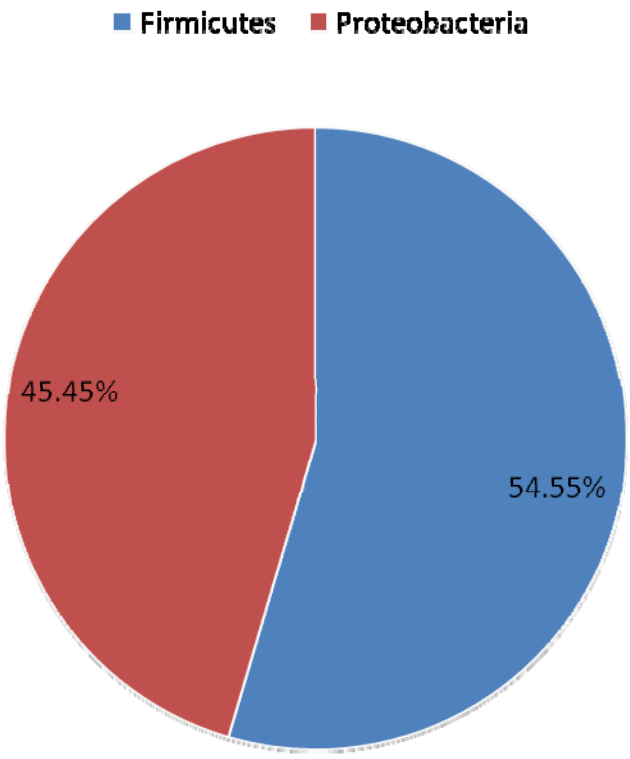

FIGURE 15. Phylogenetic analysis of the metagenomic content of soda determined by pyrosequencing.

Dinner one

Dinner One consisted of a pepperoni pizza, a salad and water. Pyrosequencing analysis of the foods in Dinner One showed a total of 21,679 sequences, 20,463 that were used for further analysis for identification of genera present. Of the sequences rejected from analysis, 1,212 came from Cyanobacteria. A phylogenetic analysis limited to Firmicutes, Proteobacteria and Bacteroidetes showed Dinner One consisted of 94.6% Firmicutes, 5.38% Proteobacteria and $1.96x10^{-4}$% Bacteroidetes (Fig. 16). A person would be consuming $2.53x10^{8}$ sequences of bacteria in 771.8 g of food if they ate this meal with the serving sizes suggested below.

Pepperoni pizza

Pyrosequencing analysis of pepperoni pizza one showed a total of 12,699 sequences, 12,685 that were used for further analysis for identification of genera present (Table 13). Of the sequences rejected from analysis, 11 came from Cyanobacteria. A phylogenetic analysis limited to Firmicutes and Proteobacteria showed pepperoni pizza consisted of 99.85% Firmicutes, 0.15% Proteobacteria (Fig. 17). There were no Bacteroidetes found on pepperoni pizza. A 125g slice of pizza would contain $7.94x10^{7}$ sequences of bacteria.

Salad

The salad analyzed consisted of iceberg lettuce, carrots, broccoli, onions, tomatoes, cheddar cheese, fried chicken tenders and buttermilk ranch. Pyrosequencing analysis of salad showed a total of 7,479 sequences, 6,765 that were used for further analysis for identification of genera present (Table 14). Of the sequences rejected from analysis, 714

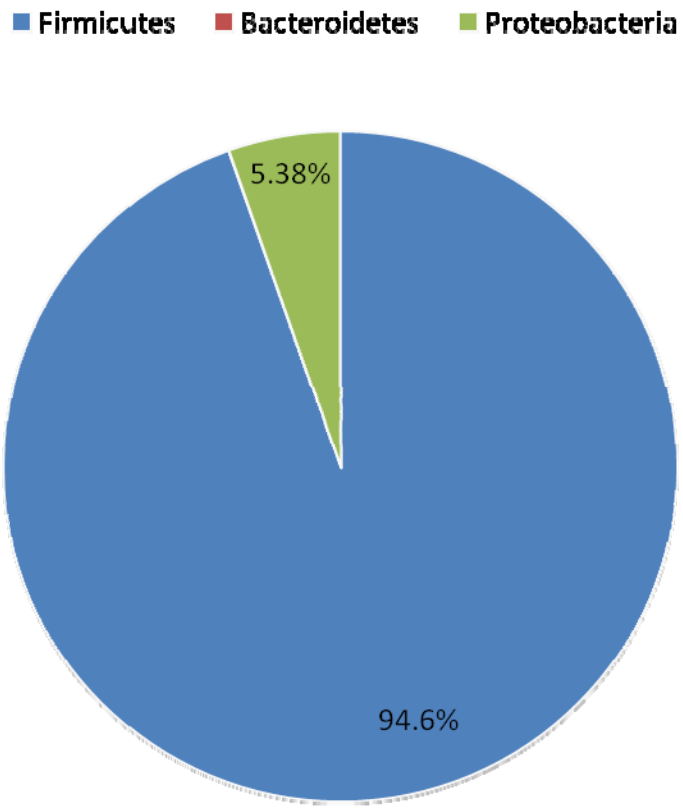

FIGURE 16. Phylogenetic analysis of the metagenomic content of dinner one determined by pyrosequencing.

TABLE 13. Results of pyrosequencing analysis of pepperoni pizza.

Genus	***Phylum***	***Number of Sequences***
Acinetobacter spp.	*Proteobacteria*	5
Anoxybacillus spp.	*Firmicutes*	13
Clostridium spp.	*Firmicutes*	0
Delftia spp.	*Proteobacteria*	7
Helicobacter spp.	*Proteobacteria*	1
Lactobacillus spp.	*Firmicutes*	4501
Lactococcus spp.	*Firmicutes*	39
Pantoea spp.	*Proteobacteria*	1
Pediococcus spp.	*Firmicutes*	33
Pseudomonas spp.	*Proteobacteria*	1
Streptococcus spp.	*Firmicutes*	8080
Tuberoidobacter spp.	*Proteobacteria*	2
Vibrio spp.	*Proteobacteria*	2
Total		**12685**

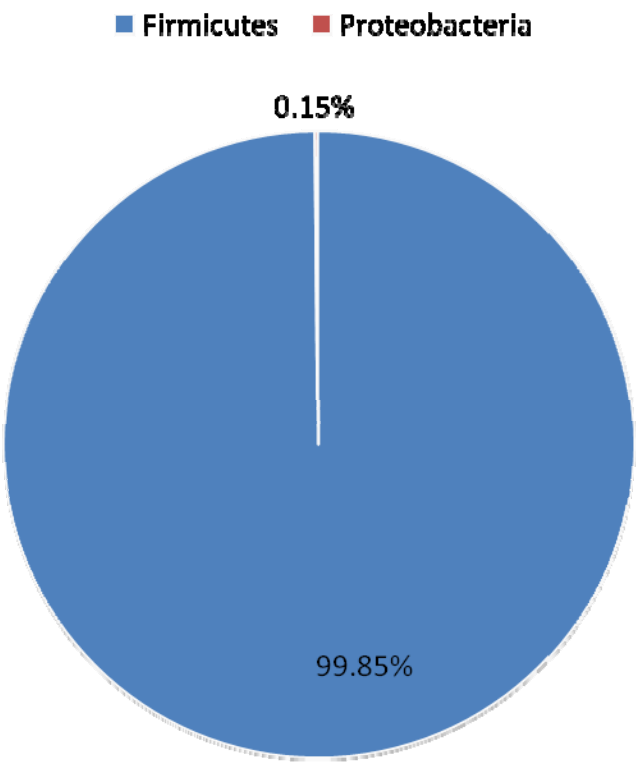

FIGURE 17. Phylogenetic analysis of the metagenomic content of Pepperoni Pizza determined by pyrosequencing.

TABLE 14. Results of pyrosequencing analysis of salad.

Genus	***Phylum***	***Number of Sequences***
Acinetobacter spp.	*Proteobacteria*	6
Carnobacterium spp.	*Firmicutes*	1
Duganella spp.	*Proteobacteria*	2
Erwinia spp.	*Proteobacteria*	7
Exiguobacterium spp.	*Firmicutes*	1
Flavobacterium spp.	*Bacteroidetes*	3
Janthinobacterium spp.	*Proteobacteria*	43
Klebsiella spp.	*Proteobacteria*	6
Lactobacillus spp.	*Firmicutes*	60
Lactococcus spp.	*Firmicutes*	5659
Leuconostoc spp.	*Firmicutes*	6
Massilia spp.	*Proteobacteria*	1
Pantoea spp.	*Proteobacteria*	3
Pectobacterium spp.	*Proteobacteria*	198
Pseudomonas spp.	*Proteobacteria*	683
Rahnella spp.	*Proteobacteria*	8
Serratia spp.	*Proteobacteria*	49
Staphylococcus spp.	*Firmicutes*	1
Streptococcus spp.	*Firmicutes*	13
Sulfitobacter spp.	*Proteobacteria*	1
Syntrophorhabdus spp.	*Proteobacteria*	1
Turicibacter spp.	*Firmicutes*	1
Vagococcus spp.	*Firmicutes*	1
Yersinia spp.	*Proteobacteria*	11
Total		**6765**

came from Cyanobacteria. A phylogenetic analysis limited to Firmicutes, Proteobacteria and Bacteroidetes showed salad consisted of 84.89% Firmicutes, 15.06% Proteobacteria and 4.43×10^{-4}% Bacteroidetes (Fig. 18). This salad weighs 303g and contains 1.57×10^{8} sequences of bacteria.

Water

The water analyzed was the same as the water in breakfast one and lunch one.

Dinner two

Dinner two consisted of a cheeseburger, fries and soda. Pyrosequencing analysis of the foods in dinner two showed a total of 16,464 sequences, 15,895 that were used for further analysis for identification of genera present. Of the sequences rejected from analysis, 557 came from Cyanobacteria. A phylogenetic analysis limited to Firmicutes, Proteobacteria and Bacteroidetes showed dinner two consisted of 98.79% Firmicutes, 1.09% Proteobacteria and 0.001% Bacteroidetes (Fig. 19). This typical fast food meal contains a total of 1.74×10^{8} sequences of bacteria in 594.8 g of food.

Cheeseburger

The cheeseburger analyzed consisted of white buns with sesame seeds, American cheese, lettuce, pickles, onions and special sauce. Pyrosequencing analysis of the cheeseburger showed a total of 15,716 sequences, 15,662 that were used for further analysis for identification of genera present (Table 15). Of the sequences rejected from

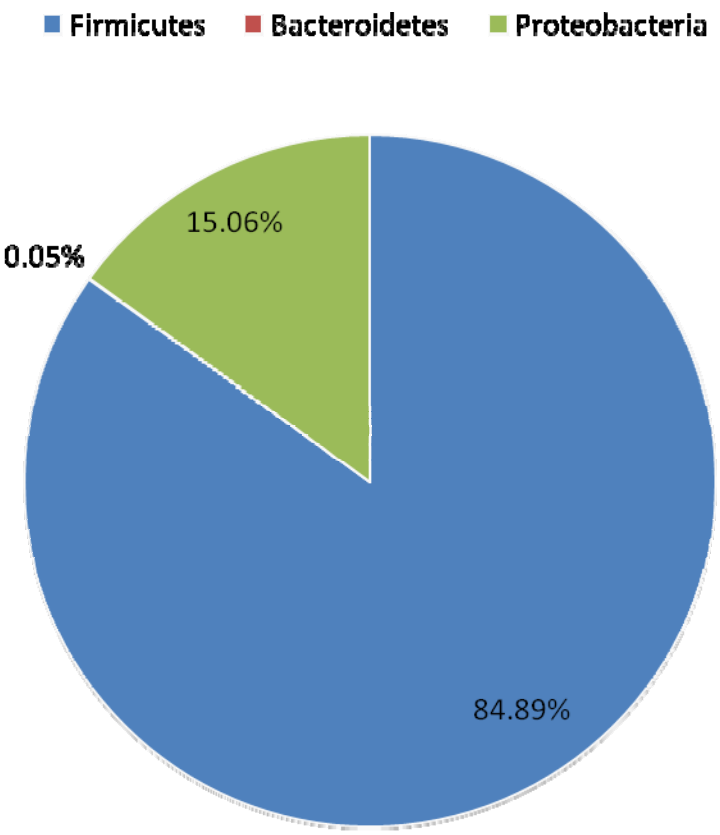

FIGURE 18. Phylogenetic analysis of the metagenomic content of salad determined by pyrosequencing.

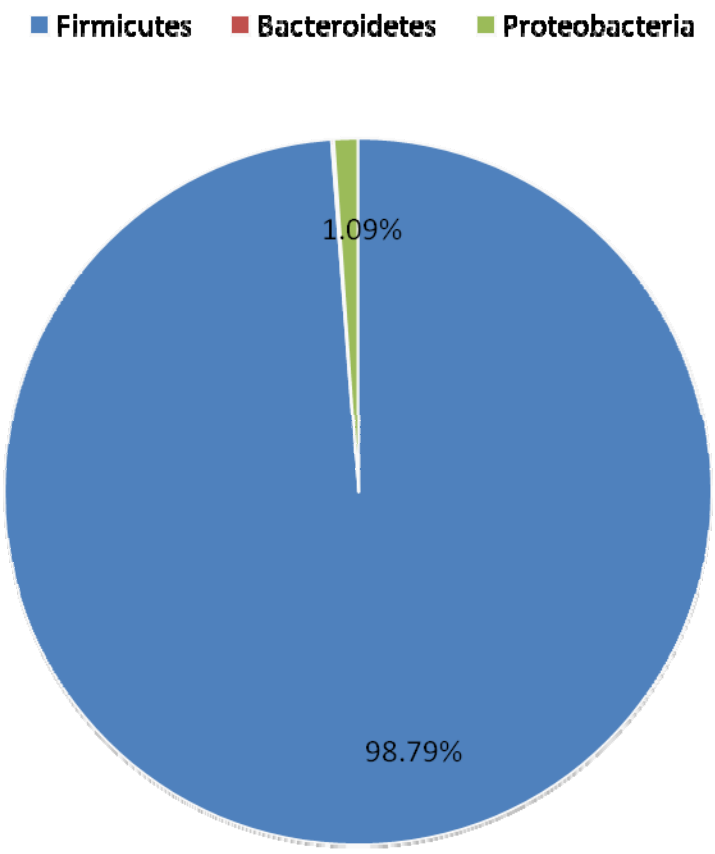

FIGURE 19. Phylogenetic analysis of the metagenomic content of dinner two determined by pyrosequencing.

TABLE 15. Results of pyrosequencing analysis of cheeseburger.

Genus	*Phylum*	*Number of Sequences*
Algibacter spp.	*Bacteroidetes*	2
Anoxybacillus spp.	*Firmicutes*	4
Bacillus spp.	*Firmicutes*	2
Bacteroides spp.	*Bacteroidetes*	1
Citrobacter spp.	*Proteobacteria*	1
Clostridium spp.	*Firmicutes*	1
Enterobacter spp.	*Proteobacteria*	1
Enterococcus spp.	*Firmicutes*	4
Flavobacterium spp.	*Bacteroidetes*	1
Hafnia spp.	*Proteobacteria*	1
Lactobacillus spp.	*Firmicutes*	103
Lactococcus spp.	*Firmicutes*	4289
Leuconostoc spp.	*Firmicutes*	4
Leucothrix spp.	*Proteobacteria*	1
Megamonas spp.	*Firmicutes*	1
Nereida spp.	*Proteobacteria*	1
Pantoea spp.	*Proteobacteria*	15
Parabacteroides spp.	*Bacteroidetes*	3
Pediococcus spp.	*Firmicutes*	1
Pelomonas spp.	*Proteobacteria*	1
Prevotella spp.	*Bacteroidetes*	1
Pseudomonas spp.	*Proteobacteria*	23
Serratia spp.	*Proteobacteria*	2
Staphylococcus spp.	*Firmicutes*	1
Streptococcus spp.	*Firmicutes*	11178
Yersinia spp.	*Proteobacteria*	20
Total		**15662**

analysis, 50 came from Cyanobacteria. A phylogenetic analysis limited to Firmicutes, Proteobacteria and Bacteroidetes showed the cheeseburger consisted of 99.53% Firmicutes, $4.0x10^{-3}$% Proteobacteria and 5.0 $x10^{-4}$% Bacteroidetes (Fig. 20). A cheeseburger weighs about 214g and contains $1.68x10^{8}$ sequences of bacteria.

Fries

Pyrosequencing analysis of the fries showed a total of 729 sequences, 222 that were used for further analysis for identification of genera present (Table 16). Of the sequences rejected from analysis, 507 came from Cyanobacteria. A phylogenetic analysis limited to Firmicutes, Proteobacteria and Bacteroidetes showed the fries consisted of 48.85% 48.85% Firmicutes, 46.60 % Proteobacteria and 4.55% Bacteroidetes (Fig. 21). A medium order of French fries weighs about 154g and has a total of $5.61x10^{6}$ sequences of bacteria.

Soda

The soda analyzed was the same as the soda lunch two.

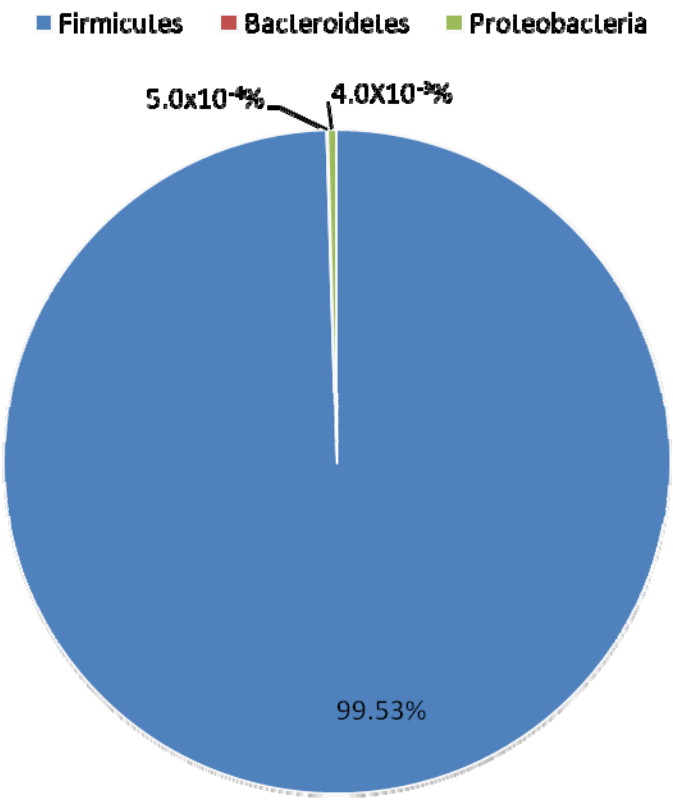

FIGURE 20. Phylogenetic analysis of the metagenomic content of cheeseburger determined by pyrosequencing.

TABLE 16. Results of pyrosequencing analysis of fries.

Genus	*Phylum*	*Number of Sequences*
Anoxybacillus	*Firmicutes*	9
Bacillus	*Firmicutes*	32
Chryseobacterium	*Bacteroidetes*	8
Clostridium	*Firmicutes*	1
Diaphorobacter	*Proteobacteria*	32
Jeotgalicoccus	*Firmicutes*	4
Lactobacillus	*Firmicutes*	9
Lactococcus	*Firmicutes*	35
Loktanella	*Proteobacteria*	1
Methylobacter	*Proteobacteria*	1
Methylobacterium	*Proteobacteria*	1
Pelomonas	*Proteobacteria*	27
Pseudomonas	*Proteobacteria*	33
Riemerella	*Bacteroidetes*	3
Stenotrophomonas	*Proteobacteria*	8
Streptococcus	*Firmicutes*	18
Total		**222**

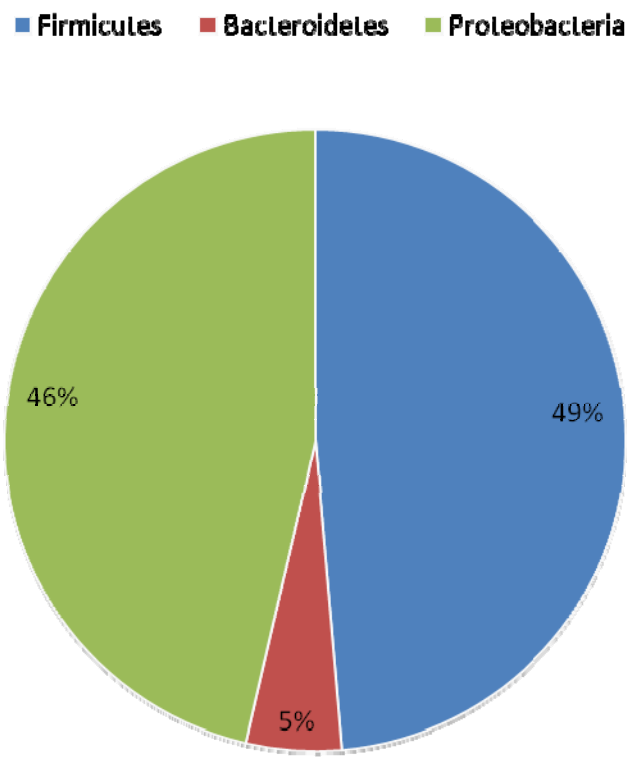

FIGURE 21. Phylogenetic analysis of the metagenomic content of Fries determined by pyrosequencing.

Discussion

Culture-based methods

Plating was included in this project to illustrate that food samples contain aerobic and anaerobic bacteria loads that are alive in these foods at time of ingestion. Including anaerobic analysis is important because many anaerobic bacteria are facultative or obligate anaerobes that cannot survive in the presence of oxygen. Of the foods that showed growth most of them had aerobic and anaerobic loads that were similar. The only one that was different was the bagel, which showed no anaerobic growth at all. The differences could have come from the specific environments that the foods create for bacteria to grow. The bagel just may have not been an environment anaerobic bacteria were capable to survive in. In this study, TSA was used to culture aerobic bacteria and BBA was used to culture anaerobic bacteria. Both media are general media that support the majority of organisms to grow. Because of this characteristic of the media, the results from plating are a good representative of the bacterial loads in the foods. Also, because the BBA plates were incubated in an aerobic hood all types of anaerobic bacteria had an optimal environment to grow in since the presence of oxygen is excluded from the hood. The entire bacterial load cannot be estimated just from culture-based methods because many bacteria are unable to grow under laboratory conditions. It is possible that the different food processing conditions could have made the bacterial cells into what could be termed as viable but unculturable. This may be why foods such as Easy Mac, water, soda, and cereal showed no growth because until analysis they were in

closed containers from their manufacturers. All other foods had to be prepared and would have multiple chances for exposure to external bacterial contamination before the consumer can purchase it. Although culture based-methods may not reflect the entire bacterial load present on the foods at any time, they do represent bacteria present in the foods and alive at the time of ingestion. The results from culture-based methods revealed that each food product contained a unique bacterial load, which did support one of my hypotheses.

Bacteroidetes and firmicutes

One objective of this study was to determine if Bacteroidetes and Firmicutes were present if foods that college students typically eat. The presence of these bacteria in the gastrointestinal tract was found by other investigators to be correlated with obesity which made us wonder if these bacteria were also found in foods we eat. The results of this study and previous studies done in Dr. Pillai's laboratory provide strong evidence that our hypothesis was correct (15). All foods contained a diverse amount of Firmicutes and most also contained Bacteroidetes. Foods that did not contain any Bacteroidetes were banana, apple, macaroni & cheese, turkey sandwich and coke. A majority of the foods were dominated by Firmicutes, which included cereal, breakfast burrito, coffee, salad, pepperoni pizza, cheeseburger, macaroni & cheese and water. Proteobacteria dominated the rest of the foods, which is another bacteria phylum that contains gut microbes (6). These foods were banana, bagel, apple, turkey sandwich and carrots. The fries and coke were almost half Firmicutes and Proteobacteria but both had a few more

Firmicutes. None of the foods were dominantly Bacteroidetes; however, the carrots showed the most significant amount of Bacteroidetes among all the foods analyzed.

Human gut flora identified in pyrosequencing data

Out of the nine phyla found among the foods only four contain microbes typically found in the human gut. These phyla are Bacteroidetes, Firmicutes, Proteobacteria and Actinobacteria (6). Specific genera of gut microbes found in these phyla were identified in the foods (Table 17). The gut inhabitants include organisms from *Bacteroides spp.*, *Clostridium spp.*, *Ruminococcus spp.*, *Enterobacter spp.*, *Lactobacillus spp.*, and *Proteus spp.* (6). Gut inhabitants included in the Actinobacteria phylum such as *Bifidobacterium spp.* and *Propionibacterium spp.* were not found in any of the foods so Actinobacteria is excluded from the rest of the discussion. Interestingly, *Escherichia spp.,* found in Proteobacteria, a major gut microbe and health contaminant was not found in any of the foods either. These findings are important because consumption of these foods could affect the diversity of gastrointestinal tract microflora. However, these studies do not prove that these bacteria will colonize in the gut. Some bacteria may not be able to survive the harsh conditions of the passageway to the gut. Further studies are needed to know if bacteria found in food can colonize in the gastrointestinal tract.

TABLE 17. Genera of gut microbes found in food.

Food Product	Bacteroidetes	Firmicutes	Proteobacteria
Apple	None	None	None
Bagel	None	*Lactococcus spp.* *Streptococcus spp.*	None
Banana	None	*Lactococcus spp.* *Streptococcus spp.*	None
Breakfast Burrito	*Prevotella spp.*	*Lactobacillus spp. Lactococcus spp.* *Streptococcus spp.*	*Proteus spp.*
Carrots	*Bacteroides spp.*	*Enterococcus spp.* *Clostridium spp.*	None
Cereal	*Bacteroides spp.*	*Lactobacillus spp.* *Streptococcus spp.*	None
Cheeseburger	*Bacteroides spp. Prevotella spp.*	*Enterococcus spp. Lactococcus spp.* *Streptococcus spp.*	*Enterobacter spp.*
Coffee w/ Half & Half	*Bacteroides spp. Prevotella spp.*	*Clostridium spp.* *Enterococcus spp.* *Lactobacillus spp. Lactococcus spp.* *Ruminococcus spp.* *Streptococcus spp.*	*Enterobacter spp.*
Coke	None	None	None
Fries	None	*Lactococcus spp.* *Streptococcus spp.*	None

TABLE 17. Continued.

Food Product	Bacteroidetes	Firmicutes	Proteobacteria
Macaroni & Cheese	None	*Lactobacillus spp.* *Lactococcus spp.* *Streptococcus spp.*	None
Pepperoni Pizza	None	*Lactobacillus spp.* *Lactococcus spp.* *Streptococcus spp.*	None
Salad	None	*Lactobacillus spp.* *Lactococcus spp.* *Streptococcus spp.*	None
Turkey Sandwich	None	*Lactobacillus spp.* *Lactococcus spp.* *Streptococcus spp.*	None
Water	*Bacteroides spp.*	*Clostridium spp.* *Lactobacillus spp.* *Lactococcus spp.* *Streptococcus spp.*	None

Correlation between nutritional content and bacterial load

There was no correlation between nutritional content like carbohydrates, total fat or calories and the kinds of bacteria present in the food (data not included). One of my original hypotheses was that there may be a higher presence of Firmicutes on high fat foods like the cheeseburger. I thought there may be a correlation because a high level of Firmicutes has been found to be a contributor to obesity just like fast food and other high fat meals. Although there were high levels of Firmicutes in the cheeseburger it cannot be correlated to the nutritional content of the burger.

Estimate of the annual consumption of bacteria

This study used foods which are part of a typical diet of a college student. A person would most likely eat a variety of foods but, the annual bacterial consumption was estimated using only the foods that were included in this study. If a college student ate three meals everyday for a year they would consume about $1.67x10^{11}$ sequences of bacteria. From admission to a university to the time of graduation a student will consume around $6.75x10^{11}$ sequences of bacteria. This is an enormous amount of bacteria and one can see how the bacteria we consume could have affect on our health.

Source of identified microorganisms

Sources of bacteria would vary greatly for each food. For instance, foods like the macaroni & cheese, coke, cereal, carrots and water were in containers until they were ready to be analyzed. Bacteria could have been introduced into the foods during

processing. The apple, bagel and banana were not in containers but didn't need to be cooked or prepared in anyway. Bacteria in these foods could have come from pre or post harvest of the foods, transportation and handling of the foods once they reached the store. All the rest of the foods needed to be prepared before they could be sold. So bacteria in these foods could have come from the kitchen and staff in addition to processing and transportation. It would be difficult to pinpoint exactly when bacteria enter these foods given the diversity of the possible sources.

Future research

This study focused on the foods typically consumed by college students but in a very small scope. Additional studies are needed to see if other foods that college students eat have similar results. Studies are also needed to look at foods we eat from infancy to adulthood. We greatly change the foods we choose to eat throughout life and this study only reflects a small part of the picture. Once we have a better understanding of the bacteria contained in foods we can try to find a correlation between the foods we eat and bacterial colonization of the gastrointestinal tract. We need to know if a person consumes a food with a known amount of bacteria causes that person to colonize those bacteria. With this information we can better understand the possible correlation of bacteria in our food and obesity.

CHAPTER IV

SUMMARY

1. **The culture based methods showed there are both aerobic and anaerobic bacteria loads in foods.** The aerobic loads ranged from 1.0CFU/g to $1.1x10^6$ CFU/g and anaerobic loads ranged from 3CFU/g to $2.6x10^6$ CFU/g. The bagel showed aerobic bacteria but no anaerobic bacteria. The foods that showed no culturable bacterial load were pepperoni pizza, coke, apple, cereal, macaroni and cheese, orange juice, and water.
2. **Metagenomic analysis of foods by pyrosequencing showed that each food contained a unique bacterial population.** When comparing all the foods that were analyzed, nine different bacterial phyla were found: Firmicutes, Nitrospirae, Fusobacteria, Actinobacteria, Bacteroidetes, Proteobacteria, Tenericutes, Cyanobacteria, and Deinococcus-Thermus. Firmicutes, Bacteroidetes, Proteobacteria and Cyanobacteria were found in the highest proportion among all the foods. Each food contained different phyla and different types of bacteria in each phylum.
3. **A college student consumes $1.67x10^{11}$ sequences of bacteria annually.** If a student eats three of the meals used in this study everyday for a year they will consume $1.67x10^{11}$ sequences of bacteria on average. After four years in school they would have consumed $6.75x10^{11}$ sequences of bacteria.

REFERENCES

1. **Bäckhed, F.** 2009. Changes in intestinal microflora in obesity: cause or consequence? Journal of Pediatric Gastroenterology and Nutrition. **48**:S56-S57.

2. **Cani, P. D., Possemiers, S., Van de Wiele, T., Guiot, Y., Evevard, A., Rottier, O., Geurts, L., Naslain, D., Neyrinkck, A., Lambert, D. M., Muccioli, G.G., and Deizenne, N.M.** 2009. Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2 driven improvement of gut permeability. Gut. **58**:1091-1103.

3. **Chang, D., Smalley, D. J., and Conway, T.** 2002. Gene expression profiling of Escherichia coli growth transitions: an expanded stringent response model. Mol. Mirco. **45**(2):289-306.

4. **Cox, M. E., Kohr, R. J. and Samia, C. K.** 1997. Comparison of quality control results with use of anaerobic chambers versus anaerobic jars. Clin. Infect. Dis. **25** (2):S137-S138.

5. **Duncan, S. H., Lobley, G. E., Holtrop, G., Ince, J., Johnstone, A. M., Louis, P., and Flint, H. J.** 2008. Human colonic microbiota associated with diet, obesity and weight loss. Intl. J. Obes. **32**:1720-1724.

6. **Eckburg, P. B., Bik, E. M., Bernstein, C. N. , Purdom, E., Dethlefsen, L.,**

Sargent, M., Gill, S. R., Nelson, K. E., and Relman, D. A. 2005. Diversity of the human intestinal microbial flora. Science. **308**:1635-1638.

7. **Gill, S. R., Pop, M., DeBoy, R., Eckburg, P. B., Turnbaugh, P. J., Samuel, B. S., Gordon, J. I., Relman, D. A., Fraser-Liggett, C. M., and Nelson, K. E.** 2006. Metagenomic analysis of the human distal gut microbiome. Science Mag. **312**:1355-1359.

8. **Guo, X., Xia X., Tang, R., Xhou, J., Zhao, H. and Wang.** 2008. Development of a real-time PCR method for *Firmicutes* and *Bacteroides* in faceces and its application to quantify intestinal population of obese and lean pigs. The Soc. for App. Micro. **47**:367-373.

9. **Heird, W. C.** 2007. Progress in promoting breast-feeding, combating malnutrition, and composition and use of infant formula, 1981-2006. J. Nutr. **137**:499S-502S.

10. **Huse, S. M., Huber, J. A., Morrison, H. G., Sogin, M.L., and Welch, D.M.** 2007. Accuracy and quality of massively parallel DNA pyrosequencing. Genome Biology **8**:R143.1-143.9.

11. **Kaur, I. P., Kuhad, A., Garg, A., and Chopra, K**. 2009. Probiotics: delineation of prophylactic and therapeutic benefits. J Med Food. **12** (2):219-235.

12. **Ley, R. E., Bäckhed, F., Turnbaugh, P., Lozupone, C. A., Knight, R.D., and Gordon, J. I.** 2005. Obesity alters microbial gut ecology. Proc. Natl. Acad. Sci. U.S.A. **102**:11070-11075.

13. **Ley, R. E., Turnbaugh, P. J., Klein, S., and Gordon, J. I.** 2006. Human gut microbes associated with obesity. Nature. **444**:21-28.

14. **Liu, Z., Lozupone, C., Hamady, M., Bushman, F. D., and Knight, R.** 2007. Short pyrosequencing reads suffice for accurate microbial community analysis. Nuc. Acids Res. **35:**(e120)1-10.

15. **Mc Elhany, K. G**. 2008. Foodborne sources of bacteria associated with obesity. Texas A&M University Undergraduate Scholars Thesis.

16. **Reinhardt, C., Reigstad, C. S., and Bäckhed, F.** 2009. Intestinal microbiota during infancy and its importance for obesity. J Ped Gastro Nutr. **48**:249-256.

17. **Roesch, L. F. W., Fulthrope, R. R., Riva, A., Casella, G., Hadwin, A. K. M., Kent, A. D., Daroub, S. H., Camargo, F. A. O., Framerie, W. G., and Triplett, E.** 2007. Pyrosequencing enumerates and contrasts soil microbial diversity. ISME **1**(4):283-290.

18. **Ronaghi, M.** 2001. Pyrosequencing sheds light on DNA sequencing. Genome Research. **11**:3-11.

19. **Tilg, H., Moschen, A. R., and Kaser, A.** 2009. Obesity and the microbiota. Gastroenterology. **136**:1476-148.

20. **White, J. R., Nagarajan, N., and Pop, M.** 2009. Statistical methods for detecting differentially abundant features in clinical metagenomic samples. PLOS Computational Biology. **5**(4):(e1000352)1-11.

APPENDIX A

ULTRA CLEAN™ SOIL DNA ISOLATION KIT INSTRUCTIONS

Alternative Protocol (For maximum yields)

1. To the 2ml Bead Solution tubes provided, add 0.25-1gm of soil sample
2. Gently vortex to mix
3. **Check Solution S1**. If S1 is precipitated heat solution to 60ºC until dissolved before use.
4. Add 60µl of Solution S1 and invert several times or vortex briefly.
5. Add 200µl of Solution IRS (Inhibitor Removal Solution). Only required if DNA is to be used for PCR.
6. Secure bead tubes horizontally using the Mo Bio Adapter tube holder for the vortex or secure tubes horizontally on a flat-bed vortex pad with tape. Vortex at maximum speed for 10 minutes.
7. Make sure the 2ml tubes rotate freely in your centrifuge without rubbing. Centrifuge tubes at 10,000 x g for 30 seconds. **CAUTION**: Be sure not to exceed 10,000 x g or tubes may break.
8. Transfer the supernatant to clean micro centrifuge tube (provided).
9. **Note**: With 0.25mg of soil and depending on soil type, expect between 400 to 450µl of supernatant. Supernatant may still contain some soil particles.
10. Add 250µl of Solution S2 and vortex 5 sec. Incubate 4°C for 10 min.
11. Centrifuge the tubes for 1 minute at 10,000 x g.
12. Avoiding the pellet, transfer entire volume of supernatant to a clean micro centrifuge tube (provided).
13. Add 1.3ml of Solution S3 to the supernatant (careful, volume touches rim of tube) and vortex for 5 seconds.
14. Load approximately 700µl onto a spin filter and centrifuge at 10,000 x g for 1 minute. Discard the flow through, add the remaining supernatant to the spin filter, and centrifuge at 10,000 x g for 1 minute. Repeat until all supernatant has passed through the spin filter. **Note**: A total of three loads for each sample processed is required.
15. Add 50µl of Solution S5 to the center for 30 seconds at 10,000 x g.
16. Discard the flow through.
17. Centrifuge again for 1 minute.
18. Carefully place spin filter in a new clean tube (provided). Avoid splashing any Solution S4 onto the spin filter.
19. Add 50µl of Solution S5 to the center the white filter membrane.
20. Centrifuge for 30 seconds.
21. Discard the spin filter. DNA in the tube is now application ready. No further steps are required.

We recommend storing DNA frozen (-20ºC). Solution S5 contains no EDTA.

APPENDIX B

FOOD PRODUCT NUTRITIONAL INFORMATION

Honey Bunches of Oats with Almonds ™		
Serving Size: 32g	Calories: 130	Calories from Fat: 25
Total Fat: 2.5g	Saturated Fat: 0g	Fiber: 2g
Total Carbohydrate: 25g	Sugars: 6g	Protein: 2g
Banana		
Serving Size: 1 cup	Calories: 200	Calories from Fat: 6
Total Fat: 1g	Saturated Fat: 0g	Fiber: 6g
Total Carbohydrate: 51g	Sugars: 28g	Protein: 2g
Bagel		
Serving Size: 1 piece	Calories: 146	Calories from Fat: 8
Total Fat: 1g	Saturated Fat: 0g	Fiber: 1g
Total Carbohydrate: 29g	Sugars: 3g	Protein: 6g
Breakfast Burrito		
Serving Size: 141 g	Calories: 320	Calories from Fat: 180
Total Fat: 17g	Saturated Fat: 6g	Fiber: 2g
Total Carbohydrate: 29g	Sugars: 2g	Protein: 13g
12th Man Brew Coffee w/ Half & Half ™		
Serving Size: 8oz	Calories: 41	Calories from Fat: N/A
Total Fat: 3.5g	Saturated Fat: 2.2g	Fiber: 0g
Total Carbohydrate: 1.3g	Sugars: 0g	Protein: 1.2g
Which Wich Turkey Sandwich ™		
Serving Size: 1 sandwich (250g)	Calories: 505.6	Calories from Fat: 217.8
Total Fat: 17g	Saturated Fat: 3.4g	Fiber: 1.1g
Total Carbohydrate: 59g	Sugars: 5.9g	Protein: 33g
Baby Carrots		
Serving Size: 3oz	Calories: 35	Calories from Fat: 0
Total Fat: 0g	Saturated Fat: 0g	Fiber: 2g
Total Carbohydrate: 8g	Sugars: 5g	Protein: 1g
Coca Cola Classic Coke ™		
Serving Size: 12oz	Calories: 140	Calories from Fat: 0
Total Fat: 0g	Saturated Fat: 0g	Fiber: 0g
Total Carbohydrate: 39g	Sugars: 39g	Protein: 0g

Easy Mac ™		
Serving Size: 58g	Calories: 220	Calories from Fat: 35
Total Fat: 4g	Saturated Fat: 2.5g	Fiber: <1g
Total Carbohydrate: 39g	Sugars: 5g	Protein: 6g
Jonagold Apple ™		
Serving Size:1 apple	Calories: 72	Calories from Fat: 0
Total Fat: 0g	Saturated Fat: 0g	Fiber: 3g
Total Carbohydrate: 19g	Sugars: 14g	Protein: 0g
Pepperoni Pizza		
Serving Size: 125g	Calories: 370	Calories from Fat: 135
Total Fat: 15g	Saturated Fat: 6g	Fiber: 3g
Total Carbohydrate: 37g	Sugars: 6g	Protein: 20g
Chick-fil-a Chicken Salad ™ w/ Buttermilk Ranch Dressing		
Serving Size: 420g	Calories: 630	Calories from Fat: 350
Total Fat: 40g	Saturated Fat: 8.5g	Fiber: 4g
Total Carbohydrate: 28g	Sugars: 8g	Protein: 41g
McDonald's Big Mac ™		
Serving Size: 1 Sandwich (214g)	Calories: 540	Calories from Fat: 260
Total Fat: 29g	Saturated Fat: 10g	Fiber: 3g
Total Carbohydrate: 45g	Sugars: 9g	Protein: 25g
McDonald's French Fries ™		
Serving Size: Medium (154g)	Calories: 380	Calories from Fat: 100
Total Fat: 19g	Saturated Fat: 2.5g	Fiber: 5g
Total Carbohydrate: 48g	Sugars: 0g	Protein: 4g

APPENDIX C

COMPLETE PYROSEQUENCING RESULTS FOR EACH FOOD PRODUCT

Bagel			
Genus	**Sequences**	**Genus**	**Sequences**
Anabaena	498	*Paracoccus*	12
Aphanizomenon	3	*Pectobacterium*	13
Arthrobacter	8	*Phormidium*	9
Catonella	4	*Planktothrix*	12
Corynebacterium	3	*Prochlorococcus*	1
Delftia	1	*Pseudomonas*	46
Devosia	3	*Salmonella*	1
Endoriftia	1	*Serratia*	3
Flavobacterium	2	*Staphylococcus*	1
Gloeotrichia	975	*Stigonema*	4
Halomicronema	80	*Streptococcus*	11
Lactococcus	16	*Tuberoidobacter*	1
Leuconostoc	23	*Tychonema*	2
Merismopedia	668	*Xylophilus*	2
Paenibacillus	10	**Total**	**2428**
Pantoea	15		
Banana			
Genus	**Sequences**	**Genus**	**Sequences**
Anabaena	267	*Microcystis*	13
Aphanizomenon	12489	*Planktothrix*	35
Dermabacter	1	*Prochlorococcus*	2
Dorea	1	*Pseudomonas*	1
Gloeotrichia	259	*Staphylococcus*	2
Halomicronema	23	*Streptococcus*	1
Kozakia	3	*Thioalkalivibrio*	1
Leptolyngbya	1	**Total**	**14579**
Merismopedia	1480		
BigMac ™			
Genus	**Sequences**	**Genus**	**Sequences**

Algibacter	2	***Megamonas***	1
Anabaena	7	***Merismopedia***	43
Anoxybacillus	4	***Nereida***	1
Bacillus	2	***Pantoea***	15
Bacteroides	1	***Parabacteroides***	3
Citrobacter	1	***Pediococcus***	1
Clostridium	1	***Pelomonas***	1
Corynebacterium	2	***Prevotella***	1
Enterobacter	1	***Pseudomonas***	23
Enterococcus	4	***Serratia***	2
Flavobacterium	1	***Staphylococcus***	1
Hafnia	1	***Streptococcus***	11178
Lactobacillus	103	***Thermus***	2
Lactococcus	4289	***Yersinia***	20
Leuconostoc	4	**Total**	**15716**
Leucothrix	1		

Breakfast Burrito

Genus	Sequences	Genus	Sequences
Anabaena	4	*Merismopedia*	6
Anoxybacillus	19	*Prevotella*	1
Brevibacillus	3	*Proteus*	1
Erythrobacter	1	*Pseudomonas*	3
Exiguobacterium	1	*Staphylococcus*	1
Gloeotrichia	8	*Stenotrophomonas*	1
Halomonas	1	*Streptococcus*	1757
Lactobacillus	4	*Thermus*	2
Lactococcus	11232	*Veillonella*	1
Macrococcus	5	**Total**	**13054**
Marinobacter	3		

Carrots

Genus	Sequences	Genus	Sequences
Anabaena	1690	*Pectobacterium*	373
Bacteroides	1	*Pedobacter*	2
Buttiauxella	1	*Phormidium*	1
Enterococcus	2	*Planktothrix*	18
Erwinia	5	*Prochlorococcus*	3
Faecalibacterium	4	*Pseudomonas*	2151
Flavobacterium	144	*Rahnella*	25

Halomicronema	46	*Raoultella*	2
Janthinobacterium	24	*Serratia*	30
Klebsiella	5	*Sulfurovum*	1
Leptolyngbya	14	*Syntrophorhabdus*	2
Leucothrix	3	*Thiothrix*	1
Merismopedia	6041	*Tychonema*	1
Microcystis	6	*Yersinia*	26
Pannonibacter	4	**Total**	**10626**
Chick-fil-a Chicken Salad ™			
Genus	**Sequences**	**Genus**	**Sequences**
Acinetobacter	6	*Pantoea*	3
Anabaena	330	*Pectobacterium*	198
Carnobacterium	1	*Planktothrix*	2
Duganella	2	*Pseudomonas*	683
Erwinia	7	*Rahnella*	8
Exiguobacterium	1	*Serratia*	49
Flavobacterium	3	*Staphylococcus*	1
Halomicronema	46	*Streptococcus*	13
Janthinobacterium	43	*Sulfitobacter*	1
Klebsiella	6	*Syntrophorhabdus*	1
Lactobacillus	60	*Turicibacter*	1
Lactococcus	5659	*Vagococcus*	1
Leuconostoc	6	*Yersinia*	11
Massilia	1	**Total**	**7479**
Merismopedia	333		
Coca Cola Classic ™			
Genus	**Sequences**	**Genus**	**Sequences**
Microbacterium	1	**Staphylococcus**	6
Microvirga	1	**Xylanimonas**	7
Pelomonas	2	**Total**	**19**
Pseudomonas	2		
Easy Mac ™			
Genus	**Sequences**	**Genus**	**Sequences**
Acinetobacter	3	*Merismopedia*	2
Anabaena	1	*Pediococcus*	2
Anoxybacillus	15	*Stenotrophomonas*	4
Bacillus	1	*Streptococcus*	1355
Geobacillus	1381	*Thermus*	3

Lactobacillus	21	*Turicibacter*	1
Lactococcus	81	**Total**	**2872**
Leucothrix	2		

Fries

Genus	Sequences	Genus	Sequences
Anabaena	357	*Merismopedia*	101
Anoxybacillus	9	*Methylobacter*	1
Bacillus	32	*Methylobacterium*	1
Chryseobacterium	8	*Pelomonas*	27
Clostridium	1	*Planktothrix*	2
Diaphorobacter	32	*Pseudomonas*	33
Halomicronema	47	*Riemerella*	3
Jeotgalicoccus	4	*Stenotrophomonas*	8
Lactobacillus	9	*Streptococcus*	18
Lactococcus	35	**Total**	**729**
Loktanella	1		

Honey Bunches of Oats ™

Genus	Sequences	Genus	Sequences
Anabaena	534	*Merismopedia*	8774
Anaerococcus	1	*Microcystis*	6
Aphanizomenon	4	*Pannonibacter*	2
Bacteroides	1	*Peptostreptococcus*	2
Clostridium	1	*Phormidium*	1
Fusobacterium	2	*Planktothrix*	13
Geobacillus	5	*Prochlorococcus*	5
Gloeotrichia	1	*Pseudomonas*	4
Halomicronema	25	*Streptococcus*	65
Lactobacillus	1	*Sulfurovum*	2
Lactococcus	1	*Syntrophorhabdus*	1
Leptolyngbya	9	*Tychonema*	10
Lutibacter	1	**Total**	**9474**
Macrococcus	3		

Jonagold Apple ™

Genus	Sequences	Genus	Sequences
Anabaena	82	*Planktothrix*	4
Comamonas	46	*Pseudomonas*	59
Diaphorobacter	15	*Rubellimicrobium*	97
Halochromatium	1	*Sporobacter*	1

Merismopedia	937	*Tychonema*	2
Microcystis	1	**Total**	**1245**
12th Man Brew Coffee w/ Half & Half ™			
Genus	**Sequences**	**Genus**	**Sequences**
Achromobacter	1	*Lysinibacillus*	1
Acinetobacter	154	*Macrococcus*	17
Aerococcus	4	*Marinimicrobium*	2
Agrococcus	13	*Marinobacter*	6
Alcaligenes	2	*Marmoricola*	2
Alistipes	14	*Merismopedia*	97
Anabaena	19	*Microcystis*	1
Anaerofilum	1	*Mycoplasma*	9
Anaerovorax	22	*Nocardioides*	6
Anoxybacillus	5514	*Oceanobacter*	1
Aphanizomenon	3	*Olsenella*	1
Arthrobacter	3	*Ornithinicoccus*	1
Atopostipes	3	*Ornithinimicrobium*	5
Bacillus	28	*Paenibacillus*	9
Bacteroides	16	*Pantoea*	5
Butyrivibrio	1	*Paracoccus*	11
Candidatus Kuenenia	1	*Pectobacterium*	9
Chryseobacterium	1	*Pelomonas*	26
Citrobacter	14	*Petrimonas*	1
Clostridium	167	*Planctomyces*	1
Corynebacterium	2	*Planktothrix*	1
Dietzia	9	*Planococcus*	2
Enterobacter	2	*Prevotella*	1
Enterococcus	12	*Pseudomonas*	100
Fastidiosipila	1	*Roseburia*	6
Flavobacterium	12	*Roseinatronobacter*	4
Geobacillus	5	*Roseomonas*	4
Gracilibacter	5	*Ruminococcus*	3
Guggenheimella	14	*Sanguibacter*	1
Halomonas	2	*Sphingomonas*	15
Helcococcus	1	*Sphingopyxis*	5
Herbaspirillum	2	*Sporobacter*	2
Janthinobacterium	2	*Stenotrophomonas*	189
Jeotgalicoccus	9	*Streptococcus*	164

Kocuria	13	*Trichococcus*	11
Lactobacillus	8	*Turicibacter*	18
Lactococcus	1	**Total**	**6821**
Listeria	3		
Pepperoni Pizza			
Genus	**Sequences**	**Genus**	**Sequences**
Acinetobacter	5	*Lactococcus*	39
Anabaena	2	*Pantoea*	1
Anoxybacillus	13	*Pediococcus*	33
Conexibacter	1	*Pseudomonas*	1
Delftia	7	*Streptococcus*	8080
Fusobacterium	0	*Thermus*	2
Gloeotrichia	9	*Tuberoidobacter*	2
Helicobacter	1	*Vibrio*	2
Lactobacillus	4501	**Total**	**12699**
Water			
Genus	**Sequences**	**Genus**	**Sequences**
Acidovorax	5	*Lactococcus*	491
Anabaena	87	*Leptolyngbya*	1
Anaerococcus	7	*Leucothrix*	1
Anoxybacillus	55	*Merismopedia*	165
Aphanizomenon	21	*Methylobacterium*	6
Bacillus	35	*Microcystis*	1
Bacteroides	1	*Paenibacillus*	11
Brochothrix	1	*Pelomonas*	4
Clostridium	2	*Pseudomonas*	7
Delftia	18	*Salmonella*	7
Fusobacterium	1	*Stenotrophomonas*	12
Geobacillus	5	*Streptococcus*	318
Gloeotrichia	44	*Sulfurovum*	1
Halomicronema	17	*Syntrophorhabdus*	1
Halomicronema	168	*Thermolithobacter*	1
Klebsiella	1	*Weissella*	10
Klebsiella	2	**Total**	**1501**
Lactobacillus	14		
Which Wich Turkey Sandwich ™			
Genus	**Sequences**	**Genus**	**Sequences**
Anabaena	85	*Merismopedia*	34

Parasporobacterium	1	*Oceanimonas*	1
Pectobacterium	3	*Pantoea*	3
Pseudomonas	6	*Serratia*	1
Lactobacillus	908	*Shewanella*	61
Lactococcus	88	*Staphylococcus*	2
Leuconostoc	31	*Streptococcus*	292
Leucothrix	1	*Vibrio*	5670
Marinobacter	1	**Total**	**7188**

APPENDIX D

CALCULATIONS OF BACTERIA FOUND IN FOOD

To find the estimated amount of bacteria found in an entire food serving the following calculation was used.

$$\frac{\#\text{ of sequences}}{1\mu l} \times \frac{50\mu l}{1\text{g food in food}} \times \frac{\text{grams of food sample}}{} = \text{Total seq. of bacteria}$$

The following table shows the amounts used in the calculations.

Honey Bunches of Oats with Almonds ™	
1µl	9474 sequences
Serving Size: 32g	$1.5x10^7$ sequences
Banana	
1µl	14,579 sequences
Serving Size: 150g	$1.09x10^8$ sequences
Bagel	
1µl	2,428 sequences
Serving Size: 110g	$1.34x10^7$ sequences
Breakfast Burrito	
1µl	13,023 sequences
Serving Size: 141 g	$9.18x10^7$ sequences
12th Man Brew Coffee w/ Half & Half ™	
1µl	6,821 sequences
Serving Size: 226.8 g (8oz)	$7.74x10^7$ sequences
Which Wich Turkey Sandwich ™	
1µl	7,211 sequences
Serving Size: 250 g	$9.01x10^7$ sequences
Baby Carrots	
1µl	10,626 sequences
Serving Size: 64 g	$3.40x10^7$ sequences
Coca Cola Classic Coke ™	

1µl	19 sequences
Serving Size: 226.8 g (8oz)	$2.15x10^5$ sequences
Easy Mac ™	
1µl	2,872 sequences
Serving Size: 58g	$3.33\ x10^6$ sequences
Jonagold Apple ™	
1µl	1254 sequences
Serving Size:150g	$9.34x10^6$ sequences
Pepperoni Pizza	
1µl	12,699 sequences
Serving Size: 125g	$7.94x10^7$ sequences
Chick-fil-a Chicken Salad ™ w/ Buttermilk Ranch Dressing	
1µl	7479 sequences
Serving Size: 303g	$1.57x10^8$ sequences
McDonald's Big Mac ™	
1µl	15,716 sequences
Serving Size: 214g	$1.68x10^8$ sequences
McDonald's French Fries ™	
1µl	729 sequences
Serving Size: 154g	$5.56x10^6$ sequences

To find the total amount of bacteria consumed in a year, the following equation was used.

$$\frac{\text{\# Sequences in 3 meals}}{\text{Day}} \times \frac{\text{365 days}}{\text{year}} = \text{Annual consumption of bacteria}$$

CONTACT INFORMATION

Name:	Alexandria Joann Haselhorst
Professional Address:	c/o Dr. Suresh Pillai Department of Poultry Science MS 2472 Texas A&M University College Station, TX 77843
Email Address:	alihaselhorst@gmail.com
Education:	B.S., Nutritional Sciences, Texas A&M University, May 2010 Minor: Biology Undergraduate Research Scholar