THANATOMICROBIOME DYNAMICS: BACTERIAL COMMUNITY SUCCESSION IN THE HUMAN MOUTH THROUGHOUT DECOMPOSITION

A thesis presented to the faculty of the Graduate School of Western Carolina University in partial fulfillment of the requirements for the degree of Master of Science in Biology.

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June 2019

ACKNOWLEDGEMENTS

I would like to thank my wonderful thesis committee, Dr. Katie Zejdlik-Passalacqua and Britt Bintz, as well as my advisor, Dr. Seán O'Connell. They were always there when I needed them and were always pushing me to succeed. There are also many other professors and staff members from Western Carolina University that have assisted me with my research, including Dr. Beverly Collins, Dr. Nick Passalacqua, Misty Cope, and especially Dr. Tom Martin. I appreciate the endless hours of support that each of WCU's faculty members have given me. Also, I would like to give a big thank you to the students in the Fall 2018 General Microbiology labs and Dr. O'Connell's Senior Research students for doing such an amazing job with all of the culture work.

I would also like to thank Dr. André Comeau from the Integrated Microbiome Resource at Dalhousie University's Centre for Comparative Genomics and Evolutionary Bioinformatics for all of the insight he provided regarding the handling, processing, sequencing, and analysis of my samples.

I am deeply thankful to the donors and the families of the donors whose remains were paramount to the success of my study. Their contribution to my research and other studies like mine is invaluable and I consider myself very fortunate to have had the privilege of working with them.

I would also like to express my gratitude toward the funding sources for my graduate research, including Western Carolina University's Provost's Internal Funding Support Grant as well as WCU's Summer 2018 Graduate Research Assistantship Grant. Without these resources, my research would not have been possible.

Last, but not least, I would like to thank my family, friends, and fellow graduate students who have always supported me and cheered me on when I needed it the most.

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LIST OF ABBREVIATIONS

ADD: accumulated degree day
AT: ambiguous taxa
βME: β-mercaptoethanol
bp: base pairs
cDNA: complementary DNA
CDI: cadaver decomposition island
CDS: coding DNA sequence
CGEB: Comparative Genomics and Evolutionary Bioinformatics
COG: Clusters of Orthologous Genes database
DBHI: dilute brain heart infusion
DNB: dilute nutrient broth
DR2A: dilute Reasoner's 2 agar
DTT: dithiothreitol
EMB: eosin methylene blue medium
ET: EnteroPluri tubes
FOREST: Forensic Osteology Research Station
GO: Gene Ontology database
HTS: high-throughput sequencing
I: inconclusive
IMR: Integrated Microbiome Resource
LM: Litmus milk medium
MCA: MacConkey agar
MGM: Microbial Genomics Module
NGS: next-generation sequencing
NIA: National Institute on Aging
NIH: National Institute of Health
NOAA: National Oceanic and Atmospheric Administration
PCA: principal components analysis
Pfam: Protein Families database
PMI: post-mortem interval
R2A: Reasoner's 2 agar
R2B: Reasoner's 2 broth
rDNA: ribosomal DNA
RDP: Ribosomal Database Project
RIN: RNA Integrity Number
rRNA: ribosomal RNA
SC: Simmons' citrate medium
SIM: sulfur, indole, motility test medium
TOD: time of death
TSA: tryptic soy agar
TSIA: triple sugar iron agar
UB: uncultured bacterium

UO: uncultured organism
UniRef50: UniProt Reference Clusters 50 database
V4: hypervariable region 4
V6: hypervariable region 6
V8: hypervariable region 8
WCU: Western Carolina University

LIST OF EQUATIONS

ABSTRACT

THANATOMICROBIOME DYNAMICS: BACTERIAL COMMUNITY SUCCESSION IN THE HUMAN MOUTH THROUGHOUT DECOMPOSITION

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Research on the post-mortem human microbiome, or thanatomicrobiome, is a rapidly developing topic in the field of forensic science. To date, the study of the thanatomicrobiome has primarily been centered on utilizing the shifting bacterial communities associated with human decomposition to more effectively establish an accurate time of death. Often, this is done by sequencing the 16S rDNA of the entire community to observe the fluctuations in the composition of the community throughout decomposition, then using those community profiles to produce predictive models to determine the post-mortem interval. Given that few studies have attempted to incorporate the functional changes within these communities, the purpose of this experiment was to shed some light on the potential functions of these post-mortem microbial communities by examining not only the 16S rDNA of the community, but the entire metagenome and metatranscriptome of the community as well. As the substrate decomposes and nutrient sources are altered, it is reasonable to expect that the changing bacterial community will be accompanied by changes in the community's metabolic capabilities. This experiment also included the identification and functional characterization of 47 unique cultured isolates some of whose identities were able to be tied back to their corresponding 16S rDNA communities and whose

metabolic activities may be tied back to metatranscriptome of those communities. From this study it was likely that the thanatomicrobiome of the oral cavity was influenced by the environment (e.g., temperature, precipitation) and there were no clear patterns between the 16S rDNA community profiles and the post-mortem interval. These results suggest that the thanatomicrobiome of the mouth may not be as suitable as internal organ systems are for determining time since death. However, functional gene expression may yet reveal more useful patterns and work is ongoing in this endeavor.

CHAPTER ONE: INTRODUCTION

Time of death (TOD) is often a crucial piece of evidence in forensic investigations. The necessity for such information can range from narrowing down suspects in criminal cases to determining asset distribution in civil disputes. In all cases, it is up to the medical examiner, along with the assistance of other forensic experts, to determine the postmortem interval (PMI), or time since death. The current methods by which PMI can be determined are heavily reliant upon the evidence available at the site of the body as well as what stage of decomposition the body has reached. In the early, or fresh, stage of decay, bodies can be assessed by the subjective examination of livor mortis (pooling of blood within tissues), rigor mortis (muscular rigidity), and algor mortis (body cooling), all of which can be influenced by ambient temperature, the body's perimortem core temperature, and the circumstances of death.[1–3] The time frame for which these methods can be utilized range from one hour to a maximum of approximately four days, depending on how long rigor takes to set in.[1] Following this period, the body will enter what are collectively known as the later stages of decomposition, which include: bloating, decay (which can be divided into active and advanced decay), postdecay (or dry decay), and skeletal (or remains).[1,4] The determination for TOD for these stages often involves the examination of insects, such as flies, belonging to Calliphoridae (blow fly) and Sarcophagidae (flesh fly) families.[1] Given that these flies often aggregate within minutes of death and develop through predictable life stages that proceed at specified rates (often depending on temperature), their successional habitation of the cadaver has become one of the primary tools used by forensic investigators.[1] However, there are circumstances in which forensic entomology cannot be utilized because insects cannot gain access to the body due to placement either indoors, underground, or behind a barrier, such as in plastic bags or caskets. It can also be harder to

determine the PMI for bodies in later stages of decay because physical changes are not as rapid as in earlier stages and because insects become less useful as multiple generations of insects inhabit the body.[1] Therefore, later stages of decay can produce PMI estimates that either vary in the range of months or are incapable of being estimated at all.[1] In cases such as this, other methods of determining the PMI by using information that is inherent to the body itself would be invaluable for forensic investigators.[5,6]

While decomposition is often described in terms of stages and PMI is measured on a timescale, it can often take different lengths of time to achieve the same stage of decomposition due to many environmental factors, chiefly ambient temperature.[4] Varying temperatures can cause dramatic changes in the rate of decomposition. Higher temperatures tend to expedite the decomposition processes because these heightened temperatures also promote increased bacterial activity and insect activity as well as faster biochemical reactions.[4] This means measuring the decomposition process simply in terms of how much time has elapsed after death does not encompass what stage of decomposition the body has reached. For example, a body discovered seven days after death in the summer will have progressed much further in the decomposition process than a body with a seven-day PMI discovered in the winter. To account for these discrepancies, accumulated degree days (ADD) are often used to measure the PMI.[4,7–9] ADD is a method of reporting PMI that factors in the amount of thermal energy that has been put into a system, allowing for a measurement of PMI that can easily be compared between cadavers.[4,10] This is of the utmost importance for studies that are performed on cadavers that do not have the same sampling schedule and may not be exposed to the same environmental parameters. Theoretically, a specific amount of thermal energy input should achieve a specific state of decomposition due to decomposition's heavy reliance on temperature regardless of varying time

periods.[4,10] Therefore, if the average amount of thermal energy in two systems is equal, the resulting decomposition state should be the same even if time periods vary. ADD is calculated by sequentially adding together the average daily temperature (in Celsius) of each day throughout a period of time. ADD is also commonly used to measure the expected larval development of insects, which have a specific threshold temperature that must be met in order to grow.[10] A study by Michaud and Moreau examined the use of a threshold temperature for the calculation of ADD for decomposition progress as decomposition is suspected to halt at temperatures below 5°C, but many studies using ADD still assume a threshold of 0°C.[4,7–9]

Many recent studies have focused on utilizing the succession of bacterial communities located in/on human cadavers to construct an objective method for estimating PMI. These bacterial communities are known as the thanatomicrobiome (thanatos, Greek for death), or the post-mortem human microbiome, and have proven to be a promising area of research that is rapidly growing in popularity.[6,11] To date, studies have been conducted to assess the changes in community composition over time as well as how these communities differ between various climates and anatomical locations.[6,11] Not only have the bacterial communities of the cadaver been studied, but the communities in surrounding soil as well as the insects occupying the carrion have also been examined for their potential use in establishing both PMI and time since deposition.[6] Multiple studies have shown that the fluctuations in community composition can be used in regression modeling to create predictive algorithms to estimate PMI.[6,9] Specifically, a collaboration between John Jay College and The University of Tennessee, Knoxville created a *k*-nearest-neighbor regressor using a machine learning technique that can calculate PMI within ± 2 days, which vastly improves upon current methods.[9] In this method, Johnson et al. characterized the bacterial community by using next-generation sequencing (NGS) techniques to target the

species-specific 16S ribosomal RNA (rRNA) gene, also referred to as 16S ribosomal DNA (rDNA).[9] High-throughput sequencing (HTS) is commonly used in metagenomic studies, including The Human Microbiome Project.[12] While many studies began by using Roche 454 pyrosequencing (Roche Applied Science, Basel, Switzerland), most studies have since switched to using Illumina platforms (Illumina® Inc., San Diego, CA) such as the Illumina MiSeq.[8,9,12–24] In a study by Chakravorty et al., it was found that when sequencing 16S rDNA, the Illumina MiSeq made fewer insertions/deletions that 454 pyrosequencing and overall resulted in more usable reads after strict quality control.[25] The Illumina MiSeq is now referred to as the instrument of choice for 16S rRNA gene sequencing.[13]

The use of 16S rDNA to characterize bacterial communities has become commonplace.[6,9,11,13,15,24,25] The 16S rRNA gene works well as a genetic barcode to differentiate between taxa because it contains 9 hypervariable regions that contain enough sequence variability to differentiate between species.[25] Although no single hypervariable region is adequate to distinguish between all bacterial species, many thanatomicrobiome studies rely on the use of hypervariable region 4 (V4) to determine the structure of bacterial communities.[8,9,15–17,19–23,25,26] According to a 2016 study by Yang et al., the optimal regions for species identification were a combination of V4-V6 and, further supporting this, a 2018 study by Fuks et al. determined that incorporating multiple hypervariable regions yielded a higher resolution of the bacterial community's profile than the V4 region alone.[14,27] For this study, the V6-V8 hypervariable regions were used. A 2015 study performed by Tremblay et al. showed that amplicons of the V6-V8 regions produce a reduction in the observed taxa when compared to V4 regions, which was hypothesized to be due to higher conservation of this area of the 16S gene.[13]

This suggests that the communities described in this study may be more conservatively defined than by what could have been obtained had the V4 region been sequenced instead.

Studies of the thanatomicrobiome experience similar trends in the succession of bacterial communities that tend to depend on what anatomical location is being sampled, including various internal organs as well nasal and oral cavities and the ear canal.[9,11] Some studies have also focused on the bacterial communities associated with skeletal remains.[22] In a 2016 study performed by Javan et al., the buccal cavities of 27 cadavers with various PMIs were identified to possess distinct microbial communities when compared to the communities associated with internal samples, including the brain, heart, liver, spleen, and blood.[11] This same study also determined that the buccal cavity had the most consistent microbial community between biological sexes when compared to other tissues that were sampled.[11] The major bacterial phyla often associated with thanatomicrobiome communities includes Actinobacteria, Firmicutes, Proteobacteria, Bacteroidetes, Tenericutes, and Verrucomicrobia.[7,9,11,15] Within these, Actinobacteria tend to be associated with samples from the mouth except for certain Actinobacteria genera, such as *Bifidobacterium*, which are associated with the gut microbiome.[7,9,11,15] This is because multiple genera of Actinobacteria are commonly found in the healthy oral microbiome of humans.[28] Meanwhile, Firmicutes, specifically *Clostridium* spp., are often found throughout decomposition when examining internal organs.[11] Also within the Firmicutes phylum, the genus *Lactobacillus* is often associated with earlier PMI.[7,11,17] The Tenericutes phylum has been found in the oral cavity, specifically in association with the bloat stage.[15] However, the two most prevalent phyla found throughout decomposition regardless of sampling location are the Firmicutes and Proteobacteria.[11,15,17,21] This is rather unsurprising as

these phyla are also two of the most species-rich and well-characterized bacterial phyla and are closely associated with humans.[29]

However, while the structural patterns of the fluctuating thanatomicrobiome are rapidly being established, few studies have examined the activity within the community. The purpose of this study was to explore this aspect of the thanatomicrobiome by combining DNA-based methods of determining community structure (including both 16S rDNA and whole shotgun metagenomes) with RNA-based methods (community metatranscriptomics) to assess how the function of these communities also change throughout decomposition. In doing this, new information can be gathered on what factors may drive the succession of the thanatomicrobiome. 16S rDNA community profiles provide information regarding the structure of the community while shotgun metagenomic data provide insight as to what genes or potential functions are present within the community by sequencing the total DNA found within a sample. Metatranscriptomic data assists in further exploration of these potential functions by determining what genes are actually being expressed in the RNA of the community.

Theoretically, as the community and underlying substrate changes during decomposition, the metabolic functions employed by the community should also change.[19] In addition to the culture-independent genomic methods of assessment, culture-based methods of studying microbial diversity were also used in this study. The cultures should be able to provide a more direct connection to specific times of decomposition by matching the 16S rDNA of the cultures to the total 16S rDNA community profiles. These resulting links of culture data to culture-independent data should then be able to tie microorganisms to specific genes from the metagenomic data as well as expressed genes from the metatranscriptomic data. Tying the activity of bacterial isolates within a laboratory setting to their function within their natural

habitat can be difficult as these two environments are often very different from one another in terms of both nutrient and resource availability as well as interactions with other microorganisms.[30] As experimental approaches move further from the field and closer to the lab (i.e., from field experiments and the biogeochemical analysis of microbial communities to the genetics and molecular biology of pure cultures) the relevance of the observed microbial activity to the natural environment or biogeochemical process decreases.[31] In 1998, Madsen referred to this conundrum as a Heisenberg uncertainty-type principle in which it is virtually impossible to both characterize the microbial community and determine its function within a system.[31] However, advances in sequencing techniques have made closing this gap more of a possibility by using HTS to determine through 16S rDNA not only which organisms are present within an environment, but also what genes they are expressing by directly sequencing all of the RNA within a given sample.

CHAPTER TWO: MATERIALS AND METHODS

Donors and Sampling Location

Samples were collected from donated human cadavers placed at Western Carolina University's Forensic Osteology Research Station (FOREST), which is an outdoor decomposition facility located in Cullowhee, NC. Donors were refrigerated until they were delivered to the facility. Upon receipt, each body was assigned a unique identification number. The donors that were used in this study were 2018-3, 2018-4, and 2018-5 and are hereafter referred to as Donors 1, 2, and 3 respectively. Donors, their biological sex, the date of their death, and the date they were received at the facility can be found in Table 1. All donors were elderly, Caucasian, and died of natural causes. Donor 1 was a male and Donors 2 and 3 were females. Donors 1 and 3 were edentulous, but Donor 2 did have her natural teeth. Donor 1 was received with a full set of upper and lower dentures. The upper set of teeth was removed to gain access to the donor's hard palate while the lower set was left in place. Upon removal of the upper dentures, a thick white film was found to coat the hard palate and was swabbed during the first sampling event for Donor 1. Donor 3 was not received with dentures. The edentulous nature of the donors was not a concern as a 2011 study performed by Michaud and Moreau showed that the oral microbiota follow a predictable pattern throughout decomposition despite the subject's dental condition (e.g. full, partial, or edentulous).[15] Each donor was placed, unclothed, on the ground in a supine position with their mouth open and without scavenger barriers apart from the fences (a double barrier, including a wooden inner fence hiding the site and an exterior chain-link fence lined with razor wire) which enclose the facility. During placement, Donor 2 was inadvertently rolled, allowing some soil to enter her mouth.

Table 1. Donor sampling schedule for the three human subjects used in the study of microbial community succession. Accumulated degree days (ADD) were derived from Equation 1.

Donor	Gender	Date of Death	Sampling Event	ADD	Sampling Date	Insect Activity
1 (2018-3)	M	4/3/2018	1	0	4/9/2018 (Received)	0
			2	49	4/13/2018	2
			3	89	4/16/2018	1
			4	138	4/20/2018	2
			5	168	4/23/2018	0
			6	222	4/27/2018	1
			7	253	4/30/2018	0
2 (2018-4)	F	4/23/2018	1	41	5/1/2018 (Received)	1
			2	106	5/4/2018	3
			3	155	5/7/2018	2
			4	223	5/11/2018	1
			5	292	5/14/2018	1
3 (2018-5)	F	5/11/2018	1	0	5/17/2018 (Received)	0
			2	84	5/21/2018	3
			3	169	5/24/2018	2
			4	291	5/28/2018	1
			5	392	6/1/2018	0

Highlighted sampling dates denote days with observed rainfall (Figure 1). Insect activity: 0 = no insect activity, 1 = mild insect activity (only adult flies present in small quantities), 2 = moderate insect activity (maggots or flies present in low to medium quantities), 3 = heavy insect activity/active colonization of the body (maggots and adult flies present in large quantities). The activity noticed during the placement of Donor 2 refers to immediate interest of adult flies.

Sample Collection and Sampling Frequency

Samples consisted of oral swabs taken from the hard palate of the donors and were collected using sterile Puritan® Hydraflock flocked swabs with a 30mm break point and dry

transport tube (Puritan Medical Products, Guilford, ME). Samples were collected upon donor placement within the facility and throughout the decomposition process at a rate of every three to four days until five sampling events were achieved for each donor, apart from Donor 1, for which seven samples were taken. The sampling schedule for each donor can be found in Table 1.

Four swabs were collected during each sampling event to be used in a variety of analyses: 1) DNA extraction and metagenomic analysis, 2) RNA extraction and metatranscriptomic analysis, 3) culturing and isolation of individual species, and 4) a backup swab in case it was needed. All samples were immediately placed on dry ice in the field until they could be stored at -80°C. Prior to sampling, each swab was moistened with sterile molecular biology grade water, except in the cases of the first two sampling events for Donor 1. The first sampling period produced moist and viscous samples that easily clung to the swabs. However, during the second sampling event, the donor's palate had dried. Therefore, it was determined that wetting the swab would enhance sample recovery in all future collections. Oral samples were collected by thoroughly rolling the swab along the roof of the donor's mouth. Swabs intended for nucleic acid recovery and the backup swabs were placed back into their original collection tubes and immediately stored on dry ice for RNA preservation. The heads of swabs intended for culturing were broken off on-site into a 2mL microcentrifuge tube of sterile 15% glycerol/Reasoner's 2 broth (R2B). All swabs were immediately transported back to the lab and stored at -80°C until extraction or isolation. Samples were named by the donor (D) and sampling event (S). For example, the second sampling event for Donor 3 would be D3S2. Donor 3 was exposed to a noticeable amount of scavenger activity, primarily from vultures. Feathers were found near the body and upon arrival on multiple sampling days numerous vultures were seen exiting Donor 3's area of the facility.

Temperature Data Collection and Standardization

To assess the effect of temperature on decomposition, temperatures were continuously monitored on site using two iButton Thermochron® temperature data loggers (Maxim Integrated, San Jose, CA) which were placed in unzipped plastic bags inside covered iButton holders that were staked into the ground on either side of the donor's head. A number of complications resulted in only one usable iButton data set per donor. One of the iButtons for Donor 1 did not record, one of the iButtons for Donor 2 was displaced by a scavenger, and one iButton for Donor 3 was covered by the donor throughout a large portion of the sampling period. The iButtons were set to record the temperature every 30 minutes, resulting in 48 temperature records each day. These temperatures were then used to determine the exact accumulated degree day at the time of sampling. Daily temperatures can be found in Figure 1.

Calculation of Accumulated Degree Days

According to the National Oceanic and Atmospheric Administration's (NOAA) Cullowhee station, throughout the duration of the sampling period, the decomposition facility experienced temperatures spanning a range of 29°F (-1.67°C) to 90°F (32.2°C) and received a total 12.12 inches of precipitation.[32] Daily temperatures and precipitation can be found in Figure 1.

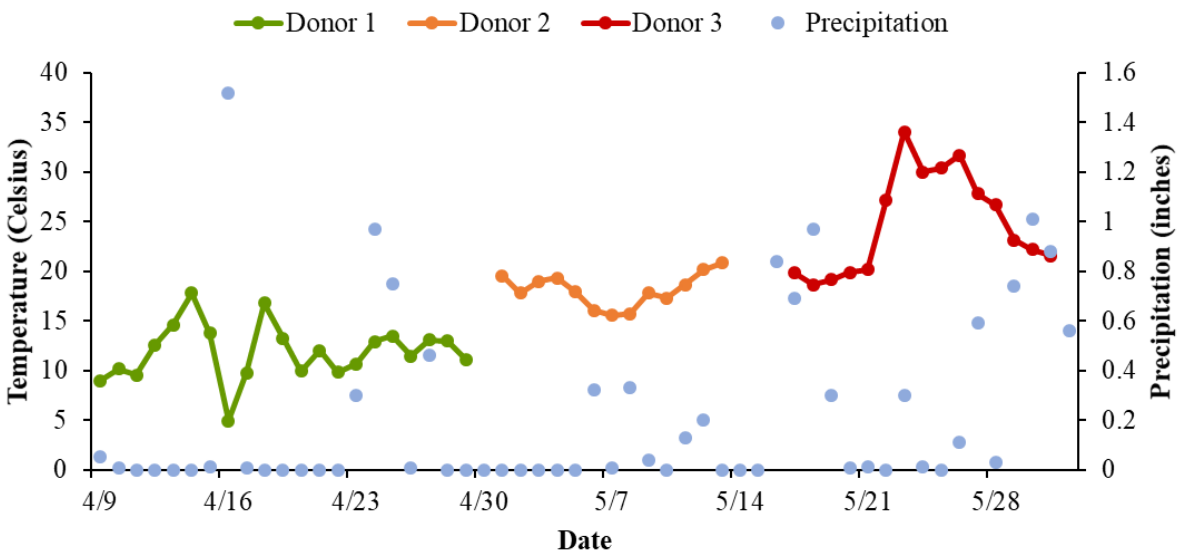

Figure 1. Average daily temperatures were recorded on site with iButton temperature loggers. Daily precipitation values were taken from Records of Climatological Observations for April-June from the NOAA station in Cullowhee, NC.[32] Values reported by NOAA as trace or "T" were graphed as 0.005 inches as the lowest reported measurement from NOAA was 0.01 inches.

This wide range of temperatures, coupled with the fact that the donors were sampled across non-overlapping time periods, meant that analyzing the data in terms of accumulated degree days was necessary. ADD was calculated for each sampling event using the formula in Equation 1.

$$ADD_t = \sum_{d=0}^{d_x-1} \bar{T} + \left(\frac{\sum_{s=0}^{t} T}{t}\right) \cdot \frac{t}{48}$$

Equation 1. Formula for calculating accumulated degree days. Variables are assigned as follows: d is the day, d_x is the day at which sampling occurred, T is the temperature in Celsius, s refers to the iButton temperature records (48 per day), and t refers to the number of temperatures that were recorded prior to sampling on a given day.

With the exception of Donor 2, the first sampling time point began at an assumed ADD of 0 due to storing the donors in refrigerators prior to delivery. In the case of Donor 2, the body was received in an early stage of decomposition and was exhibiting the beginnings of some mild skin slippage around the waist. This was due to Donor 2 being found two days after death in her home. Because of this, her initial ADD was calculated based on the National Institute of Health's (NIH) National Institute on Aging (NIA) recommendation for thermostat settings for the elderly during winter months.[33] According to the NIA, thermostats should be kept between 68-70°F to avoid hypothermia.[33] Therefore, the average, 69°F (20.5°C), was used as the average household temperature across 2 days, resulting in a baseline ADD of 41 for Donor 2. The ADD for each sampling event can be found in Table 1. The differences between the average daily temperatures and the accumulated degree days for each donor can be found in Figure 2.

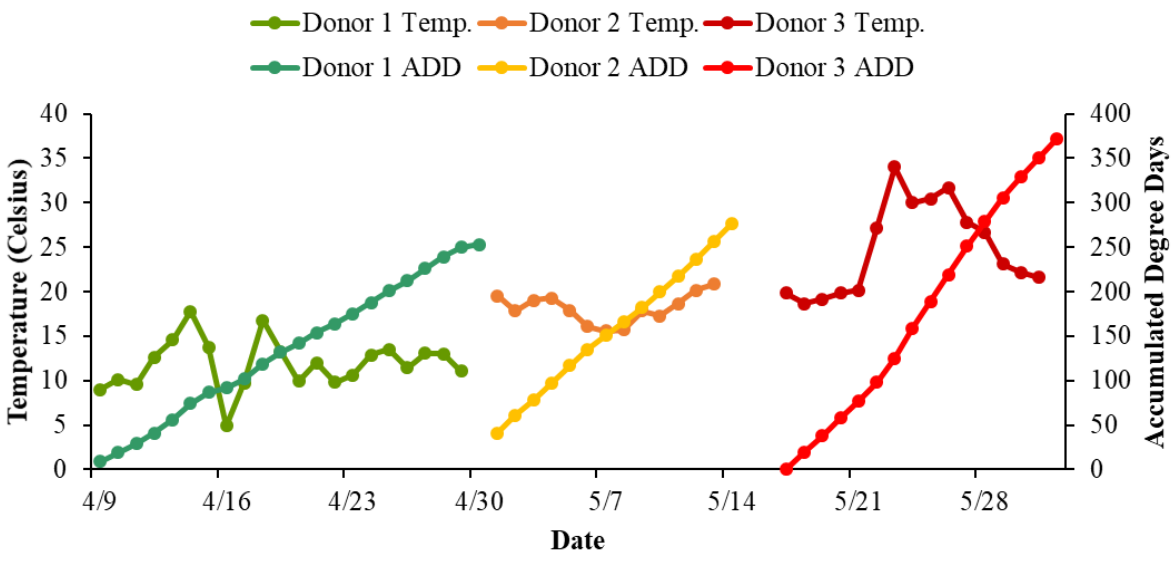

Figure 2. Differences between the accumulated degree day values and average daily temperatures experienced by each donor throughout the sampling period. Temperatures were recorded using iButton temperature loggers while ADD was calculated using Equation 1.

Sample Processing

DNA and RNA Extraction and Purification

For any samples that inadvertently collected maggots during swabbing, maggots were removed with a sterile scalpel prior to extraction. Both DNA and RNA were extracted and purified separately using QIAGEN's RNeasy® PowerMicrobiome® Kit (QIAGEN, Hilden, Germany) following the manufacturer's protocol with some minor modifications. All plasticware used for extractions was UV-sterilized prior to use. For both DNA and RNA extractions, the optional phenol/chloroform/isoamyl alcohol step was not utilized. Dithiothreitol (DTT) was used as the reducing agent in lieu of β-mercaptoethanol (βME) at a ratio of 20µL of 2M DTT per 1

mL of lysis buffer for both DNA and RNA extractions. During the third step of both DNA and RNA extractions, samples were placed in a BioSpec Mini-BeadBeater-1 (BioSpec, Bartlesville, OK) at 2500 rpm for 1 minute instead of using the recommended vortex adapter at maximum speed for 10 minutes. During RNA extraction, to prevent the copurification of small RNAs, 70% ethanol was used in place of buffer PM4 during the addition of binding salts (buffer PM3). DNA extracts were stored at -20°C while RNA extracts were stored at -80°C.

DNA and RNA Quantitation

After extraction, samples were quantified using Agilent's 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). DNA was quantified using the Agilent DNA 12000 Kit and the DNA 12000 Series II Assay following the protocol detailed in Agilent's DNA 12000 Kit Quick Start Guide (Agilent Technologies). RNA was quantified using the Agilent RNA 6000 Pico Kit and the Prokaryotic Total RNA Pico Series II Assay following the protocol detailed in Agilent's RNA 6000 Pico Kit Quick Start Guide (Agilent Technologies). DNA and RNA quantities as well as the RNA Integrity Numbers (RIN) for each sample can be found in Table 2. All samples met the 0.2 ng/μL minimum required for sequencing and all met the preferred quantity of 1 ng/μL, except sample D3S3. Sample D3S3 was concentrated using a vacuum centrifuge with no added heat in order to double its 0.71 ng/μL original quantity. After concentration, it was not economical to use the 2100 Bioanalyzer, so the sample was reassessed using a NanoDrop™ 2000 Spectrophotometer (Thermo-Fisher Scientific, Waltham, MA). However, the NanoDrop™ results were implausible as it stated that the concentration was approximately 12 ng/μL. Therefore, there is not an accurate final DNA concentration for sample D3S3 in Table 2.

Table 2. Quantitation of the DNA and RNA extracted from oral swabs as well as the cDNA synthesized from the extracted RNA. RNA Integrity Numbers (RIN) were provided by the Agilent 2100 Bioanalyzer. Higher RIN values correspond to higher quality RNA extracts.

Sample	DNA (ng/µL)	RNA (pg/µL)	RNA Integrity Number (RIN)	cDNA (ng/µL)
D1S1	41.97	43,563	2.4	3.48
D1S2	4.72	1,121	3.3	0.31
D1S3	7.51	70,303	3.9	6.3
D1S4	1.64	2,080	5.6	0.5
D1S5	10.71	14,768	6.7	3.26
D1S6	10.34	49,116	5.7	7.64
D1S7	10.97	17,123	5.1	3.38
D2S1	3.07	3,688	2.7	0.628
D2S2	1.88	5,768	4.6	1.49
D2S3	0.97	2,772	6.4	0.814
D2S4	4.83	2,488	3.1	0.634
D2S5	1.63	2,219	N/A	0.542
D3S1	3.32	2,218	5.5	0.552
D3S2	4.87	5,680	N/A	0.772
D3S3	0.79*	1,353	5.4	0.424
D3S4	4.64	1,969	4.2	0.488
D3S5	15.23	61,028	5.1	7.64

*The quantity listed for D3S3 is from prior to being concentrated.

Complementary DNA Synthesis

RNA was converted to complementary DNA (cDNA) using Thermo Fisher Scientific's SuperScript™ IV First-Strand Synthesis Kit (Thermo-Fisher Scientific) following the manufacturer's protocol. The cDNA was then quantified using Invitrogen's Qubit® 2.0 Fluorometer with the Qubit® dsDNA HS Assay Kit (Life Technologies, Carlsbad, CA)

following the protocol as described in the Qubit® 2.0 Fluorometer User Manual. The concentration of cDNA in each sample can be found in Table 2. All cDNA quantities met the requirements for sequencing.

DNA and RNA Sequencing

Amplification, library preparation, and sequencing for all samples was performed by the Integrated Microbiome Resource (IMR) lab located at Dalhousie University's Centre for Comparative Genomics and Evolutionary Bioinformatics (CGEB) in Halifax, Nova Scotia. Amplification of the 16S rDNA V6-V8 hypervariable regions was performed using bacteria specific primers (B969F: ACGCGHNRAACCTTACC and BA1406R: ACGGGCRGTGWGTRCAA). 16S rDNA samples were sequenced using the Illumina MiSeq platform while shotgun metagenomic and metatranscriptomic samples were run on an Illumina NextSeq 550 (Illumina® Inc., San Diego, CA) following an in-house protocol derived from multiple sources.[34–37]

Culturing and Isolation

Students in the 2018 fall semester Principles of General Microbiology lab (BIOL 413/513) and Senior Research class (BIOL 480) grew and isolated cultures from the swabs that had been stored in glycerol at -80°C. The forty-six BIOL 413/513 students grew their cultures on low nutrient Reasoner's 2 agar (R2A) to obtain quick growing cultures while the ten BIOL 480 students grew their cultures on a variety of diluted media to obtain slower growing cultures. Using the diluted media minimized the slower growing organisms from being outcompeted by fast growing colonies. The diluted media included 1% R2A (DR2A), 1% brain heart infusion (DBHI) agar, and 10% nutrient broth agar (DNB). Recipes for the dilute media can be found in Tables A1-A3 of Appendix A. In total, 69 isolates were grown in culture and characterized.

Examination of Isolates

The General Microbiology students worked throughout the semester gathering colony characteristics and performing both microscope-based and metabolic tests on their isolates. Cultures from the Senior Research students were simply identified via 16S rDNA sequencing. The microscope-based analyses included Gram-staining, negative staining, flagella staining, spore staining, capsule staining, and a hanging drop assay. The metabolic tests included growing the bacteria in various growth parameters including temperatures from 4°C-55°C, pH solutions from 3-10, salt solutions from 0%-15%, and in anaerobic enclosures. The students also performed a motility test, oxidase test, and catalase test on their isolates.

Other tests examined the bacteria's ability to utilize specific substrates. Due to monetary and time restrictions, not all of these tests were used for each isolate. Instead, the isolate's 16S rDNA taxonomic classification determined which tests were most appropriate for each isolate. Students checked for their isolate's ability to ferment adonitol, arabinose, dulcitol, glucose, lactose, mannitol, sorbitol, and sucrose. Seven students also used Biolog EcoPlates™ (Biolog Inc., Hayward, CA) to determine usable carbon sources for their isolates. Cultures were examined for their ability to decarboxylate lysine and ornithine and deaminate phenylalanine in EnteroPluri devices. They were also tested for their ability to hydrolyze casein, DNA, esculin, gelatin, lipids, starch, and urea. Students also tested whether their isolates could reduce nitrate and sulfur and checked for production of nitrite, ammonia, gaseous nitrogen, hydrogen sulfide, and indole. Isolates were also examined for their production of acetoin via the Vogues-Proskauer test. Isolates were grown on blood agar to determine their hemolytic abilities. Two *Lactobacillus* isolates were grown in lactic acid selective broth to assess their production of lactic acid. Metabolic tests were performed using a variety of media types including eosin methylene blue

(EMB) agar, mannitol salt agar, MacConkey agar (MCA), EnteroPluri tubes (ET), triple sugar iron agar (TSIA), litmus milk (LM), sulfur indole motility (SIM), and Simmons' citrate (SC) media. Students also tested for peptone catabolism, growth on tryptic soy agar (TSA), citrate utilization, lecithinase production, and coagulase production. Isolates were tested for resistance to amoxicillin, ampicillin, chloramphenicol, ciprofloxacin, clindamycin, colistin, erythromycin, nalidixic acid, Neosporin, nitrofurantoin, penicillin, streptomycin, and tetracycline.

Identification of Isolates

Isolates were sent to GENEWIZ (GENEWIZ Inc., South Plainfield, NJ) for 16S rDNA sequencing. GENEWIZ performed direct colony sequencing of each isolate's 16S rDNA using a Sanger sequencing approach. Each sequence was identified using the Sequence Match and Classifier tools from the Ribosomal Database Project (RDP).[38,39] Students in the General Microbiology lab then used these identifications to determine which tests would be applicable to their organism and assist them in narrowing down a taxonomic classification, if necessary. Of the 69 pure cultures, 47 unique species were identified.

Data Analysis

16S rDNA Taxonomic Profiling

The operational taxonomic units (OTUs) for the profiles of the 16S rDNA community metagenome and shotgun metagenomic sequences were determined using CGEB-IMR's bioinformatic pipeline, which utilizes the open-source platform QIIME2.[40] The raw OTU counts for each taxonomic level can be found in Appendix B.

Statistical Analysis of 16S rDNA Community Structures

Visualizations of the 16S rDNA OTU profiles were created through R v.3.6.0 using the tidyverse, reshape2, FactoMineR, Heatplus, vegan, coin, gplots, ggplot2, RColorBrewer, and

extrafont packages.[41–52] The OTU counts from CGEB-IMR were converted to proportions within each sample's community for statistical analyses. For the resulting stacked bar charts for phylum and class levels, the "Minor Contributors" designation was set to include any taxa that contributed to less than 2% of the community profile. For the levels of order, family, and genus, this classification included any taxa that represented less than 5% of the community profile. RStudio was also used to perform principal components analysis (PCA) for the samples at each taxonomic level.[42] In some cases, outliers were removed to improve the resolution of the PCA plot. Heatmaps were also generated using RStudio. The distances between samples on the heatmaps were determined using a Bray-Curtis dissimilarity matrix as well as average linkage hierarchical clustering.

CHAPTER THREE: RESULTS

16S rDNA Community Profile Assessment

Operational taxonomic units for the 16S rDNA metagenomic data varied greatly between samples (Figure 3). The lowest OTU count was for sample D3S5 (1,229 OTU) while the highest count was for sample D1S3 (8,594 OTU) (Figure 3). The average OTU count was 4,822. There did not appear to be a relationship between ADD and OTU count or donor and OTU count. A breakdown of the OTU counts at each taxonomic level can be found in the tables within Appendix B. All OTUs were able to be classified to the phylum level. In total, 7 phyla, 12 classes, 28 orders, 53 families, and 95 genera were identified. The 16S rDNA sequences only provided a high enough resolution to identify 11 unique species.

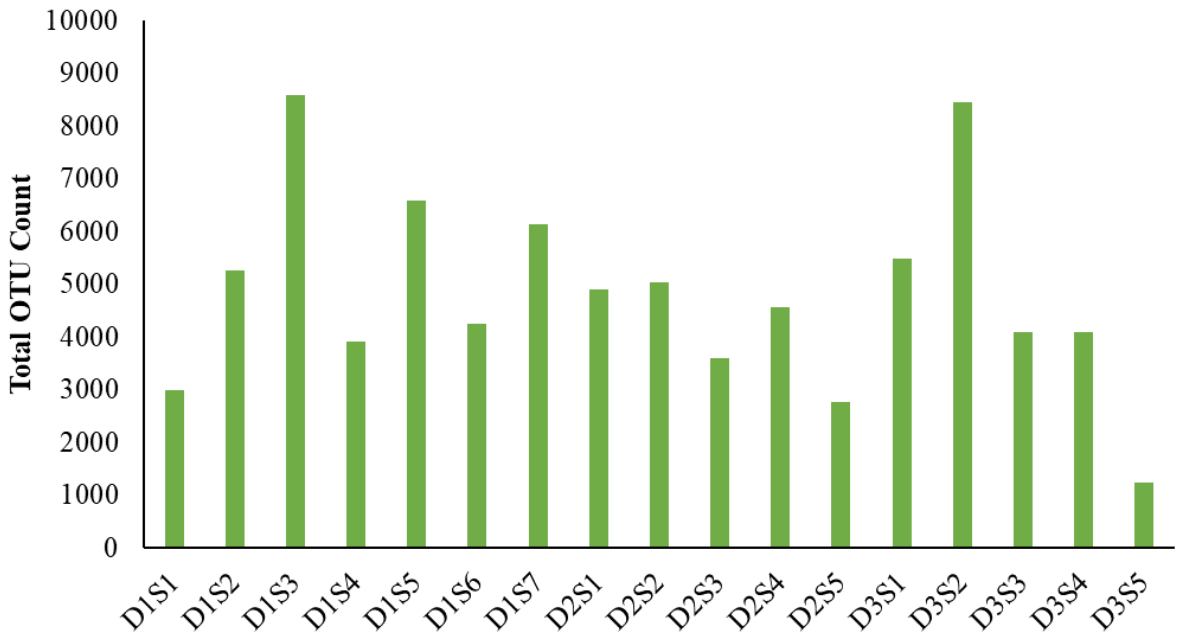

Figure 3. Total operational taxonomic unit (OTU) abundance per sample based on the output of CGEB-IMR's QIIME2-based bioinformatic pipeline for the 16S rDNA community profiles (D: donor, S: sampling event).

As demonstrated at multiple levels of taxonomy in Figures 4-8, there are noticeable shifts in community composition as ADD progresses. Sequences of 16S rDNA from the phylum Actinobacteria persist throughout the earlier ADD then drop off and resurface at ADD 291 (Figure 4). Interestingly, there is a small amount of 16S rDNA sequences from the Verrucomicrobia phylum at ADD 49 that are not found in any other samples. Throughout the majority of decomposition, Firmicutes and Proteobacteria appear to dominate. From these phyla, Gammaproteobacteria and Bacilli (Firmicutes) appear to be the key classes until Clostridia

(Firmicutes) appears at ADD 138 (Figure 5). For the phylum Fusobacteria, a minor contributor, 16S rDNA sequences were only present in sample D2S1, ADD 41 (Table B1 of Appendix B).

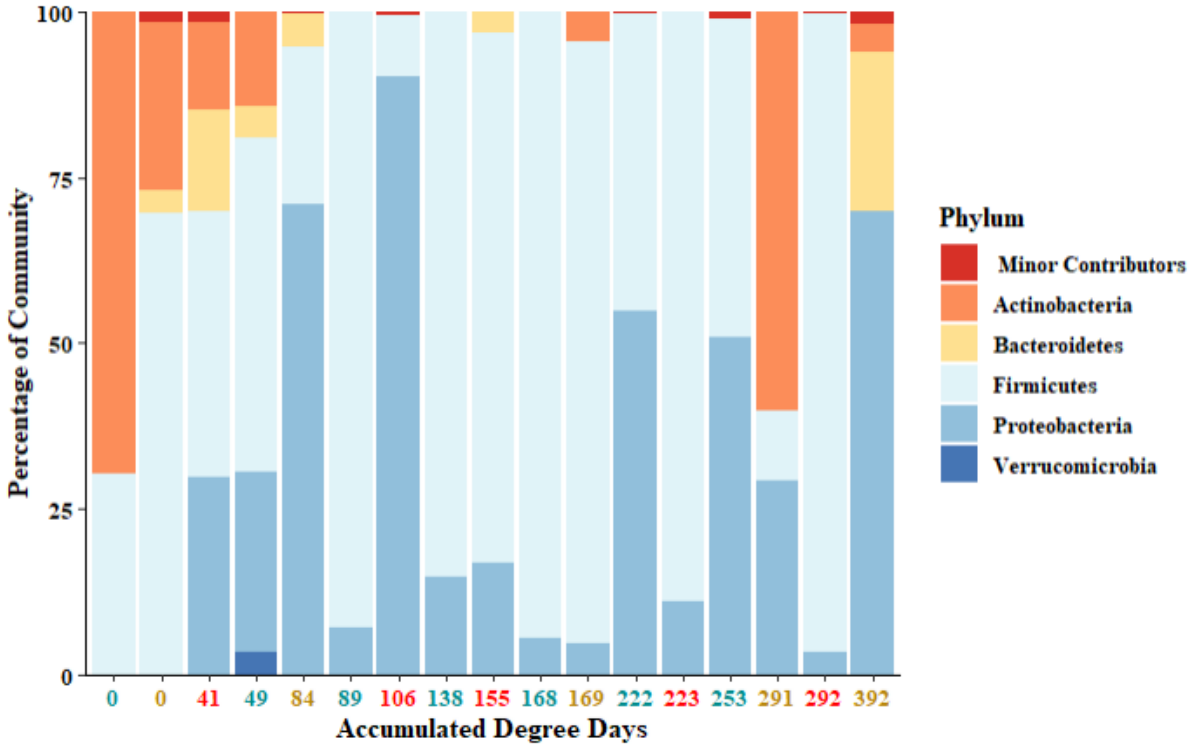

Figure 4. Distribution of phyla within the 16S rDNA community profiles throughout decomposition. The ADD labels are color coded by which donor the sample came from: Blue = Donor 1, red = Donor 2, and gold = Donor 3. For this taxonomic level, minor contributors consisted of any taxa that contributed to less than 2% of the overall community profile.

At the class level, a similar pattern emerges to that of the phylum level. 16S rDNA sequences from two main classes, Bacilli and Gammaproteobacteria, fluctuate throughout the middle of decomposition with sequences from the Actinobacteria class appearing early and late in the decomposition process (Figure 5). While some sequences belonging to the Clostridia class are present at ADD 41, they seem to primarily take hold at ADD 106 (Figure 5). According to raw OTU counts, Clostridia are present in every sample, except those with an ADD of 0. In some samples Clostridia OTU counts were low enough to be considered a minor contributor for that community's 16S rDNA profile (Figure 5, Table B2).

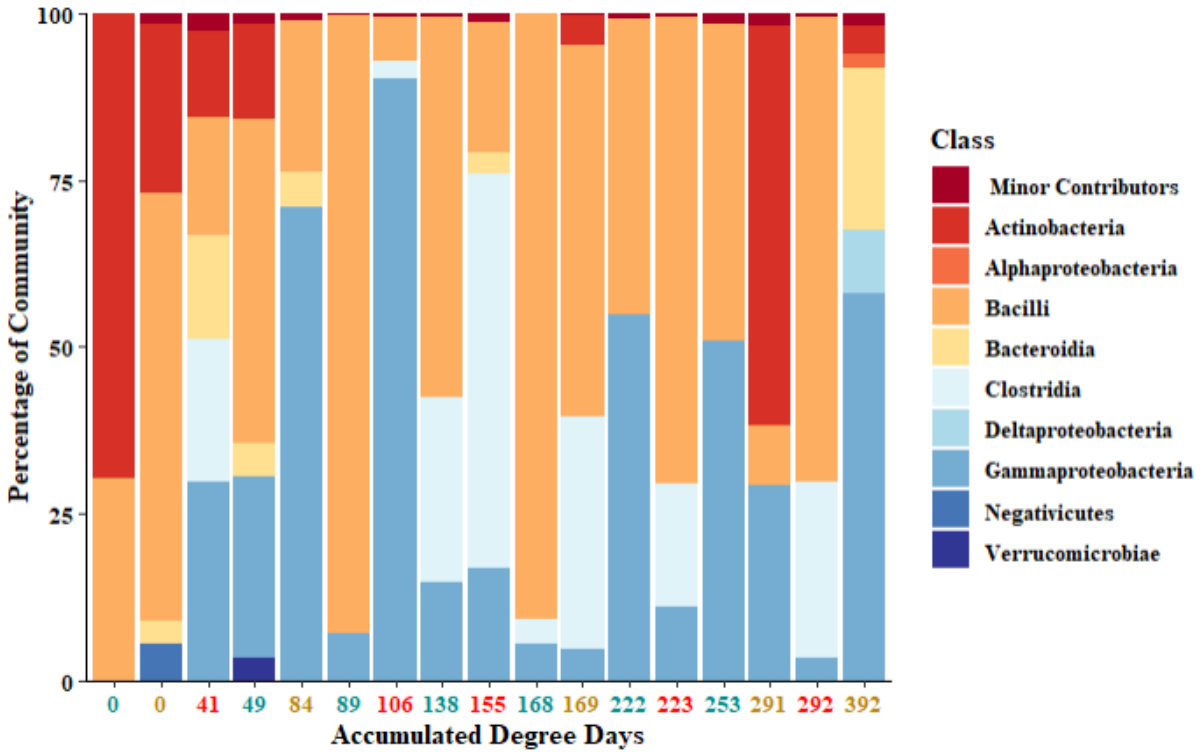

Figure 5. Distribution of classes within the 16S rDNA community profiles throughout decomposition. The ADD labels are color coded by which donor the sample came from: Blue = Donor 1, red = Donor 2, and gold = Donor 3. For this taxonomic level, minor contributors consisted of any taxa that contributed to less than 2% of the overall community profile.

At the order level, it becomes easier to resolve some of the diversity present within each sample. Notably, the first sampling time for Donor 2 (ADD 41) appears to contain more diversity than the first sampling times (ADD 0) for Donors 1 and 3 (Figure 6). Overall, no clear patterns emerge apart from a distinction between very early and mid to late decomposition. Early ADD values are primarily associated with 16S rDNA from the Actinomycetales and Lactobacillales

orders (Figure 6). At this resolution, it does become apparent that the sequences from the Actinobacteria phylum that are present at both ends of decomposition belong to different orders (Figure 6). Early decomposition contains sequences from the Actinomycetales and Micrococcales orders whereas the spike in Actinobacteria sequences experienced in later decomposition come from the Corynebacteriales order (Figure 6). Throughout the middle of decomposition, sequences belonging to the Bacillales, Clostridiales, Lactobacillales, Cardiobacteriales, and Pseudomonadales orders appear to vacillate without any apparent pattern (Figure 6). Bacillales, Clostridiales, and Lactobacillales belong to the Firmicutes phylum while Cardiobacteriales and Pseudomonadales belong to the Proteobacteria phylum.

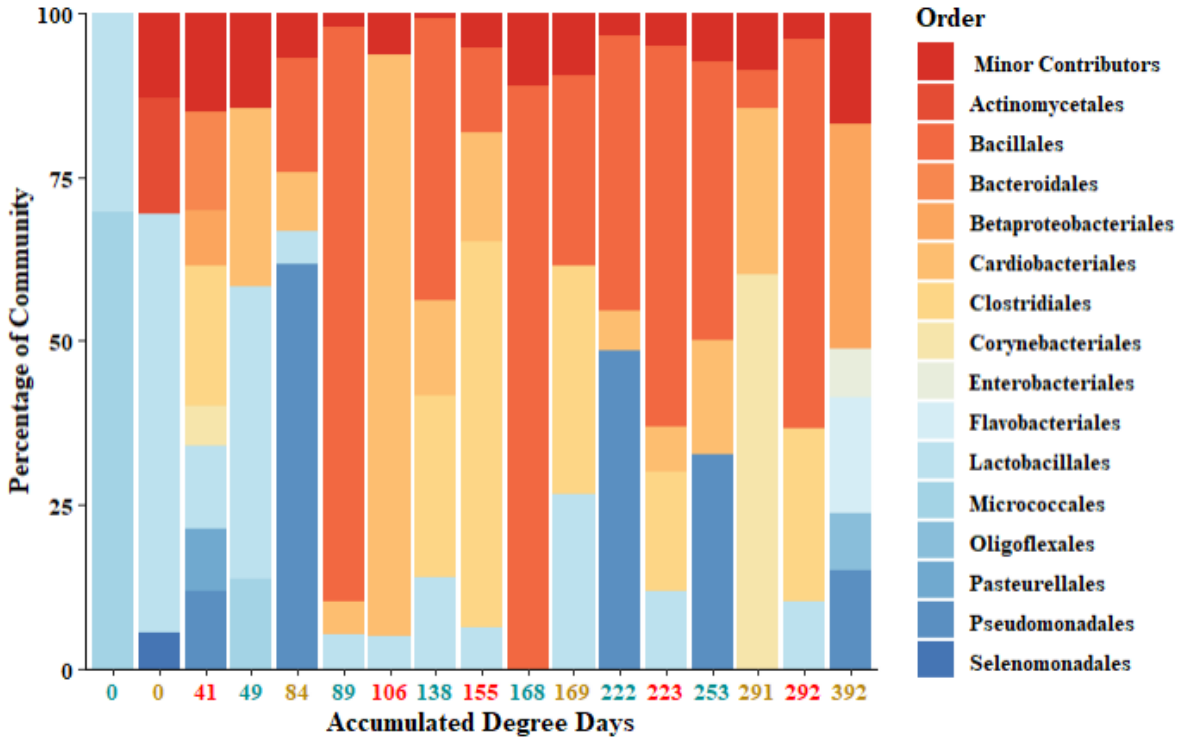

Figure 6. Distribution of orders within the 16S rDNA community profiles throughout decomposition. The ADD labels are color coded by which donor the sample came from: Blue = Donor 1, red = Donor 2, and gold = Donor 3. For this taxonomic level, minor contributors consisted of any taxa that contributed to less than 5% of the overall community profile.

The family and genus levels depict more of the same patterns experienced at higher taxonomic classifications. However, at these levels it becomes easier to assess whether or not certain taxa are typically more closely associated with human, soil, or other microbiomes. For example, Wohlfahrtiimonadaceae, a family that appears at later ADD values, is associated with flesh flies (Figure 7).[15] This family has its largest spike at ADD 106, which is the second

sampling time for Donor 2 (Figure 7). This sampling event exhibited the highest insect activity of all sampling events. Carnobacteriaceae is only found at ADD 0 for Donor 3 and ADD 41 for Donor 2 (Figure 7). Members of this family are commonly found associated with food and the human body.[53] Enterococcaceae are only found in later ADD and are often associated with the gut microbiomes of humans and animals, which often leads to this family also being associated with the soil as they are shed through defecation.[54] The members noted in this study could come from the soil or are likely associated with purged bodily fluids. While overall there appears to be a trend of families and genera more closely associated with soil becoming more prevalent as time goes on, there is no obvious visible pattern that follows a shift in specific families or genera throughout decomposition (Figures 7 and 8).

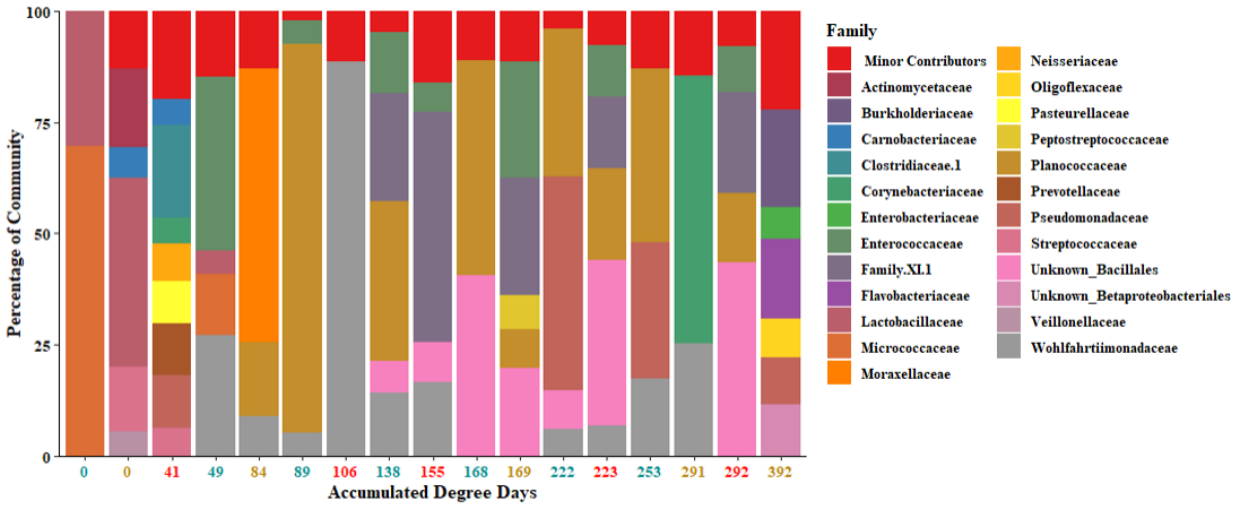

Figure 7. Distribution of families within the 16S rDNA community profiles throughout decomposition. The ADD labels are color coded by which donor the sample came from: Blue = Donor 1, red = Donor 2, and gold = Donor 3. For this taxonomic level, minor contributors

consisted of any taxa that contributed to less than 5% of the overall community profile. “Unknown_Bacillales” and “Unknown_Betaproteobacteriales” refers to sequences that could not be classified beyond those corresponding orders.

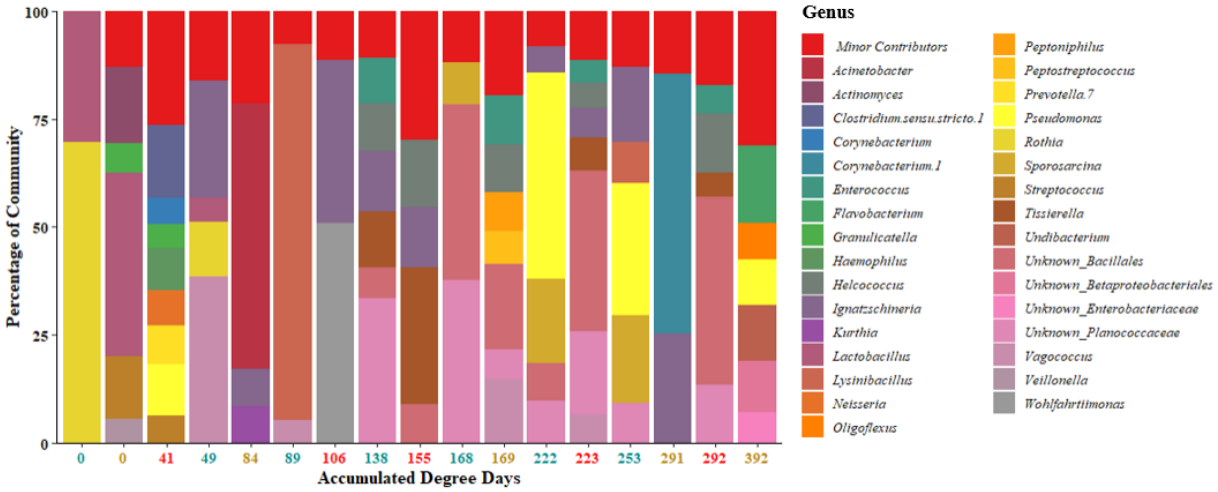

Figure 8. Distribution of genera within the 16S rDNA community profiles throughout decomposition. The ADD labels are color coded by which donor the sample came from: Blue = Donor 1, red = Donor 2, and gold = Donor 3. For this taxonomic level, minor contributors consisted of any taxa that contributed to less than 5% of the overall community profile. “Unknown_Bacillales” and “Unknown_Betaproteobacteriales” refers to sequences that could not be classified beyond those corresponding orders. “Unknown_Enterobacteriaceae” and “Unknown_Planococcaceae” refer to sequences that could not be classified beyond those corresponding families.

When examining the differences between the bacterial communities of each sample at the phylum level using principal components analysis, it is clear that most of the phyla, excluding Actinobacteria and Verrucomicrobia, had a large impact on how the samples clustered together (Figure 9). Visible clusters include samples from the ADD ranges of 101-150 and 151-200 as well as some of the later samples from ADD ranges 201-250 and 251-300 (Figure 9). At this taxonomic level, samples from Donor 1 tend to cluster together while samples from Donors 2 and 3 tend to be more evenly dispersed, indicating greater variability in their community makeup with time (Figure 9). The two main components of the PCA for the phylum level are, together, capable of explaining 52.2% of the differences between samples (Figure 9). At this level there does not appear to be a clear pattern of clustering based on ADD range. The correlation coefficient for each phylum along both dimensions 1 and 2 can be found in Table 3. The phylum with the highest positive correlation coefficient along dimension 1 is Bacteroidetes (0.852) and the phylum with the highest negative correlation coefficient along dimension 1 is Firmicutes (-0.830) (Table 3). Along dimension 2, the phylum with the highest positive correlation coefficient is Fusobacteria (0.520) and the phylum with the highest negative correlation coefficient is Proteobacteria (-0.600) (Table 3).

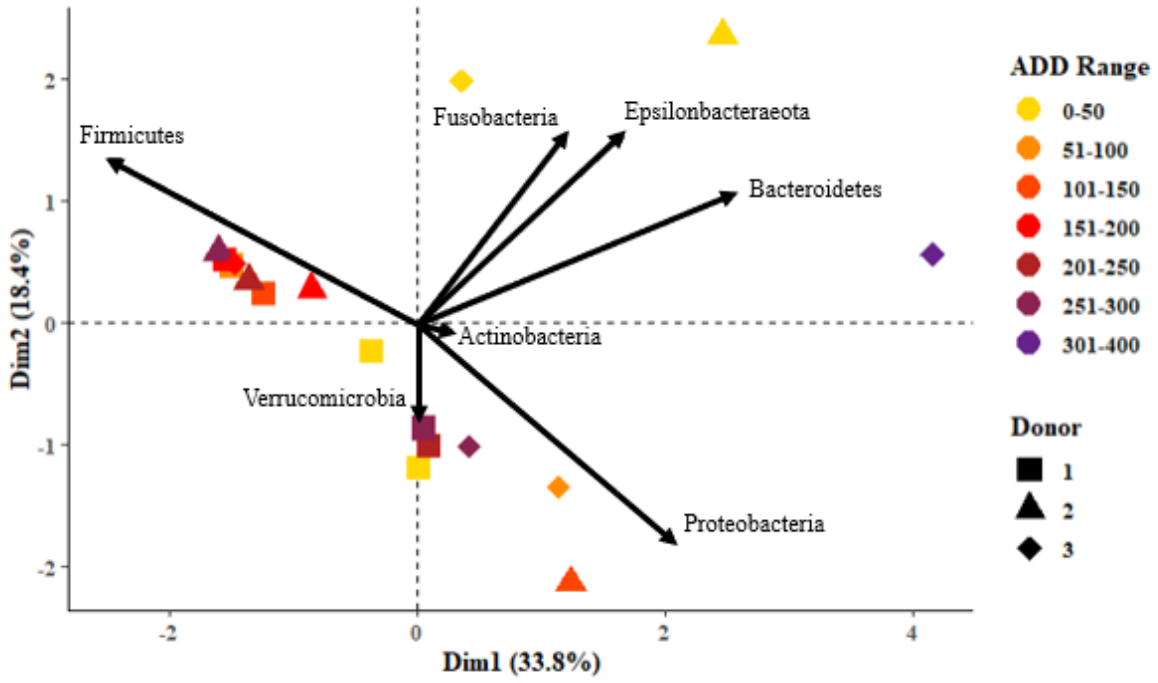

Figure 9. Principal components analysis of 16S rDNA diversity data obtained from the decomposing human remains of three donors over time at the level of phylum, including vectors indicating which phyla were most important in differentiating the samples.

Table 3. Correlation coefficients for the principal components analysis of the 16S rDNA community profiles at the phylum level.

Phylum	Dimension 1 Correlation Coefficient	Dimension 2 Correlation Coefficient
Actinobacteria	0.096	-0.025
Bacteroidetes	0.852	0.353
Epsilonbacteraeota	0.552	0.512
Firmicutes	-0.830	0.452
Fusobacteria	0.401	0.520
Proteobacteria	0.690	-0.600
Verrucomicrobia	0.002	0.264

At the class level, two samples were outliers: D2S3 (ADD 155) and D3S5 (ADD 392) (Figure 10a). When looking at overall bacterial community makeup to explain this, it is possible that these samples are considered outliers due to the large quantity of sequences belonging to the Clostridia class found in D2S3 as well as the high quantity of Bacteroides sequences found in D3S5 (Figure 5). Both with and without the outliers removed, there is a clear clustering of samples from Donor 1 (Figure 10) that was also observed at the phylum level (Figure 9). With the outliers removed, there appears to be some loose clustering associated within ADD ranges such as clusters for ranges 51-100 and 151-200, but there is still some overlap of samples from other ADD ranges (Figure 10b). In total, the class level community profiles were able to explain 50.3% of the differences between samples before the outliers were removed and 43.4% when the outliers were removed. The correlation coefficients for each class along dimensions 1 and 2 of each principal components analysis (both with and without outliers) can be found in Table 4.

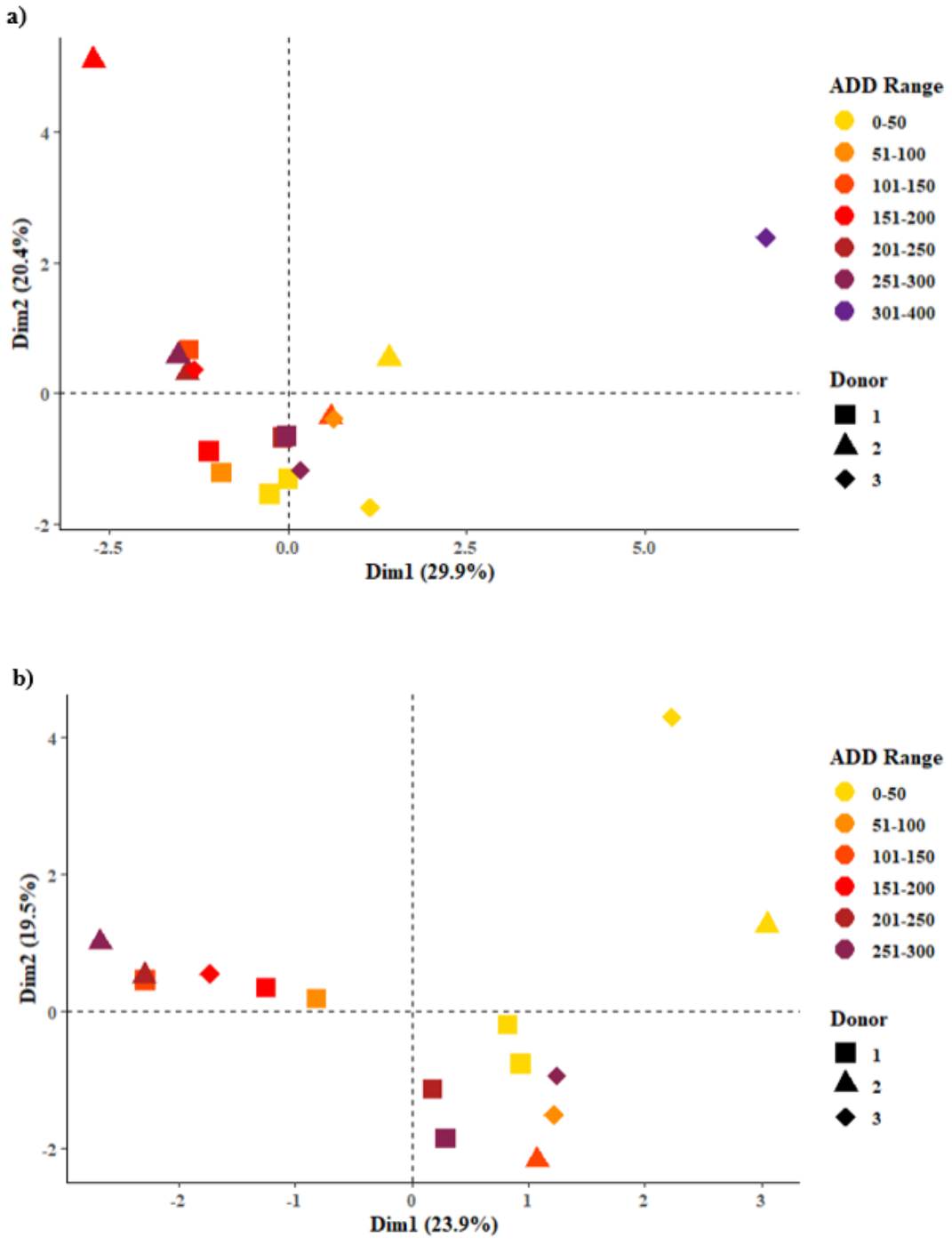

Figure 10. Principal components analysis of 16S rDNA diversity data obtained from the decomposing human remains of three donors over time at the level of class. a) original PCA

without outliers removed, b) PCA with outliers removed. The removed outliers were D2S3 (ADD 155) and D3S5 (ADD 392).

Table 4. Correlation coefficients for the principal components analysis of the 16S rDNA community profiles at the class level, including both the PCA with outliers included and the PCA with outliers removed.

	Correlation Coefficients of PCA with Outliers		Correlation Coefficients of PCA without Outliers	
Class	**Dimension 1**	**Dimension 2**	**Dimension 1**	**Dimension 2**
Alphaproteobacteria	0.848	0.365	0.046	-0.324
Actinobacteria	0.091	-0.388	0.423	0.089
Bacilli	-0.520	-0.350	-0.642	0.445
Bacteroidia	0.845	0.378	0.643	0.221
Campylobacteria	0.700	0.040	0.443	0.788
Clostridia	-0.501	0.781	-0.545	0.304
Deltaproteobacteria	0.846	0.368	0.000	0.000
Erysipelotrichia	-0.525	0.784	-0.765	0.177
Fusobacteriia	0.179	0.083	0.481	0.218
Gammaproteobacteria	0.475	0.059	0.394	-0.721
Negativicutes	0.178	-0.251	0.440	0.787
Verrucomicrobiae	0.002	-0.201	0.148	-0.133
Unspecified Firmicutes	-0.391	0.791	-0.422	0.175

For the PCA at the order level, sample D3S5 (ADD = 392) was once again found to be an outlier (Figure 11a). Prior to the removal of the outlier, the community profile was capable of explaining 52.5% of the differences observed in each sample. Once sample D3S5 was removed, this shifted to 35.3%, which is notably lower than the percent of variance that can be explained at higher taxonomic levels. Prior to the removal of sample D3S5, there is a clustering of samples

from the ADD range 0-50, that is even clearer once the outlier is removed (Figure 11). Removing sample D3S5 did assist in the resolution of the PCA. However, apart from the clear distinction between the range of 0-50 and the rest of the decomposition process, there are no clear clusters based on ADD (Figure 11b). Once again, there is a general clustering of samples taken from Donor 1 when compared to the samples from Donors 2 and 3 (Figure 11b). The correlation coefficients of each order along dimensions 1 and 2 for each PCA (with and without outliers) can be found in Table 5.

The principal components analyses for the family, genus, and species level were not included as they accounted for markedly lower explanations of the differences between samples. Without removing outliers, the explained variation for each PCA was respectively 47.4%, 39.4%, and 36.3%. Upon the removal of the outlier, S3E5 (ADD 392), in each PCA, these values changed to 37.2%, 31%, and 31.3%, respectively. For the analyses performed at these lower taxonomic levels, patterns similar to those at higher taxonomic levels were observed. Samples from Donor 1 tended to cluster while samples from Donors 2 and 3 were more scattered, indicating a wider range of diversity within these samples compared to Donor 1. Also, there was no distinguishable clustering of samples based on ADD ranges.

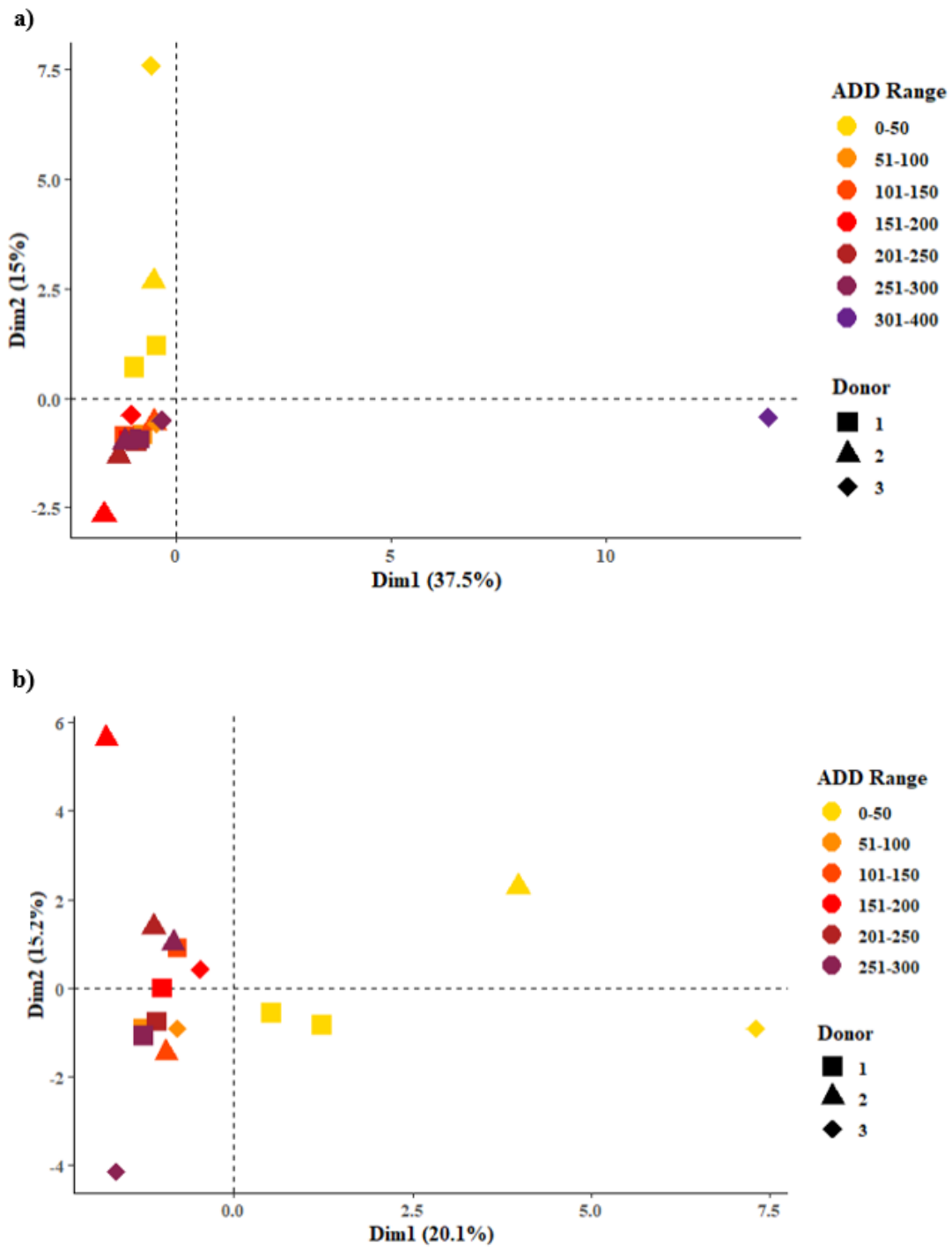

Figure 11. Principal components analysis of 16S rDNA diversity obtained from the decomposing human remains of three donors over time at the level of order. a) original PCA without outliers removed, b) PCA with outliers removed. The removed outlier was D3S5 (ADD

392). D3S1 was not removed in order to showcase the clustering of the samples with low ADD values.

Table 5. Correlation coefficients for the principal components analysis of the 16S rDNA community profiles at the class level, including both the PCA with outliers included and the PCA with outliers removed.

	Correlation Coefficients of PCA with Outliers		Correlation Coefficients of PCA without Outliers	
Order	**Dimension 1**	**Dimension 2**	**Dimension 1**	**Dimension 2**
Actinomycetales	-0.049	0.918	0.893	-0.061
Bacillales	-0.291	-0.414	-0.442	0.073
Bacteroidales	0.010	0.455	0.603	0.364
Bdellovibrionales	0.995	-0.050	0.000	0.000
Betaproteobacteriales	0.973	0.018	0.409	0.231
Bifidobacteriales	-0.041	0.864	0.811	-0.012
Campylobacterales	0.614	0.704	0.891	-0.060
Cardiobacteriales	-0.128	-0.177	-0.244	-0.259
Chitinophagales	0.995	-0.050	-1.03E-17	-9.89E-17
Clostridiales	-0.241	-0.316	-0.176	0.846
Corynebacteriales	0.010	-0.029	-0.139	-0.499
Enterobacteriales	0.947	-0.119	-0.330	-0.453
Erysipelotrichales	-0.219	-0.423	-0.312	0.803
Flavobacteriales	0.968	-0.070	-0.095	-0.130
Fusobacteriales	-0.037	0.302	0.441	0.292
Lactobacillales	-0.193	0.802	0.789	-0.064
Legionellales	0.995	-0.050	0.000	0.000
Micavibrionales	0.995	-0.050	0.000	0.000
Micrococcales	-0.066	0.175	0.158	-0.087
Oligoflexales	0.995	-0.050	0.000	0.000
Pasteurellales	-0.037	0.302	0.441	0.292
Propionibacteriales	0.789	0.051	0.148	-0.136
Pseudomonadales	0.084	-0.146	-0.151	-0.163
Rhizobiales	0.994	-0.055	-0.141	-0.138
Selenomonadales	-0.048	0.915	0.888	-0.062
Sphingobacteriales	0.995	-0.052	-0.183	-0.529
Verrucomicrobiales	-0.032	0.135	0.137	-0.108
Xanthomonadales	0.983	-0.062	-0.183	-0.528
Unknown Firmicutes	-0.142	-0.329	-0.221	0.739
Unknown Bacilli	-0.096	-0.152	-0.125	0.175
Unknown Clostridia	-0.135	-0.327	-0.218	0.745
Unknown Gammaproteobacteria	-0.093	-0.159	-0.191	-0.173

The heatmap created for the phylum level community profiles reiterated that the phyla with the highest abundances are the Firmicutes, Proteobacteria, and (to a lesser extent) Actinobacteria (Figures 4 and 12). The samples were separated using a Bray-Curtis dissimilarity matrix and according to the distribution, there does not seem to be any patterns of clustering for samples with similar ADD values (Figure 12). ADD 41 and 49 are similar to one another, but not to either 0 ADD values (Figure 12). ADD 84 and 89 were not very similar in composition (Figure 12). The community composition of ADD 291 is most closely related to ADD 0 for D1S1 (Figure 12). Some of the middle ADD values cluster together, such as 138, 155, and 168, but there are also samples with very different ADD values that cluster here as well (Figure 12). While there is a lack of clustering for ADD values, it is worth noting that there is also a lack of clustering between donors (Figure 12). This means that there were no obvious differences between the communities at the phylum level found for each donor.

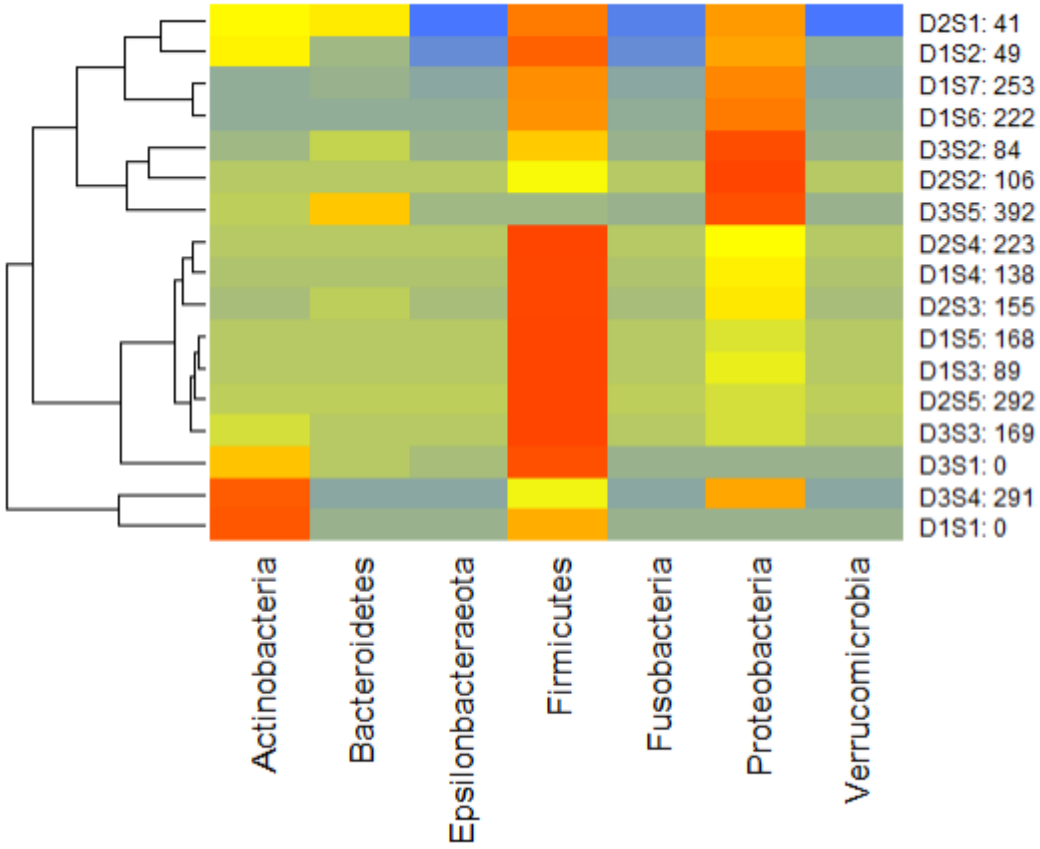

Figure 12. Heatmap of phylum abundance. On the right, samples are denoted by Sample ID: ADD (D stands for donor and S stands for sampling event). On the left, samples have been categorized using a Bray-Curtis dissimilarity matrix. High abundances correspond to red/orange coloration while low abundances correspond to blue coloration.

The class level heatmap showed similar clustering compared to the phylum level. ADD 291 was still most closely related to ADD 0 for sample D1S1 (Figure 13). However, ADD 41 and 49 were separated, likely due to the large amount of Bacilli sequences found in 49 (Figure 13). There was once again no obvious clustering based on ADD values or donor. The class level

heatmap again showed that the classes with the highest abundances throughout most community profiles were the Bacilli and Gammaproteobacteria (Figures 5 and 13).

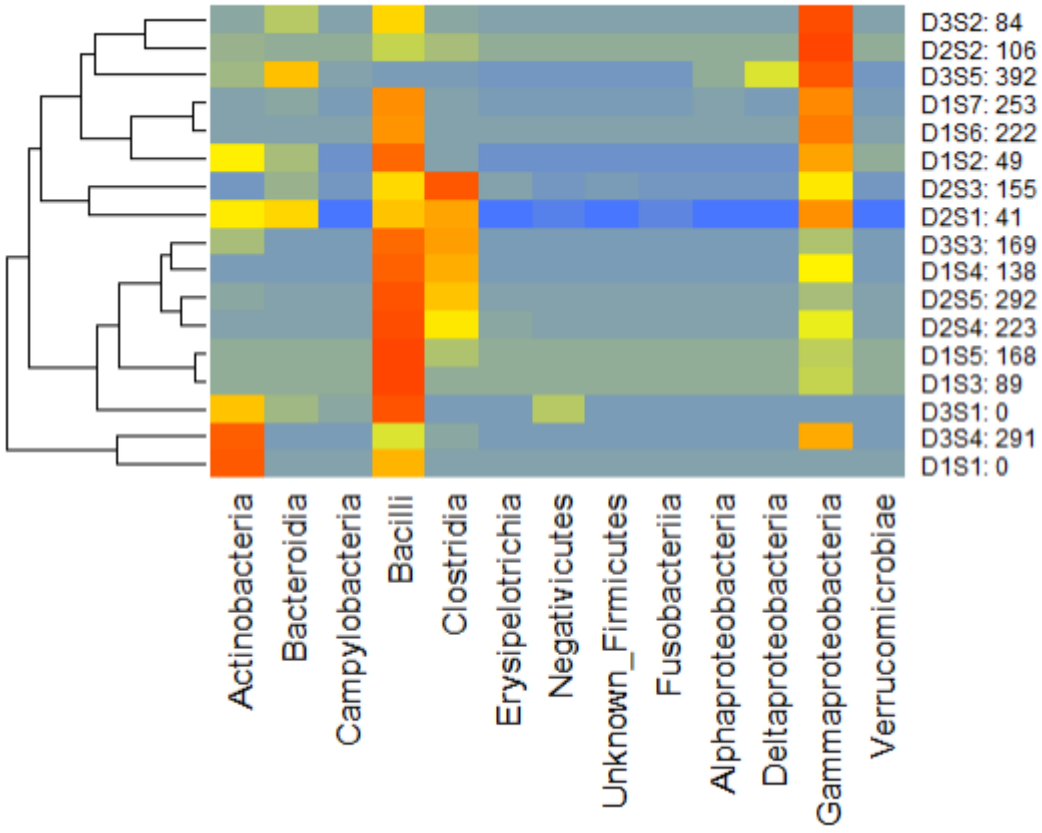

Figure 13. Heatmap of class abundance. On the right, samples are denoted by Sample ID: ADD (D stands for donor and S stands for sampling event). On the left, samples have been categorized using a Bray-Curtis dissimilarity matrix. High abundances correspond to red/orange coloration while low abundances correspond to blue coloration. “Unknown_Firmicutes” refers to sequences that could not be classified beyond the Firmicutes phylum.

Culture Results

The cultures exhibited a wide array of characteristics, environmental tolerances, and metabolic activities. The metabolic capabilities for each isolate can be found in Appendix C. Each isolate was identified by its 16S rDNA (Tables 3-5). One culture was only identified to the family level (Table 3). In total, 47 unique species were identified from the 69 pure cultures that had been characterized. Each culture belonged to one of the four main phyla that were prevalent in the 16S rDNA community profiles: Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria. A majority of the cultures (24) came from the Proteobacteria phylum while only two cultures, *Myroides profundi* and *Flavobacterium piscis*, belonged to the Bacteroidetes phylum.

Once each isolate was identified via their 16S rDNA, a corresponding match was searched for within the 16S rDNA sequences derived directly from the donors without culturing (Tables 3-5). Some isolates were matched to the same genus from the same sampling time and donor while others were matched to sequences that could only be identified to a higher taxonomic level, such as family or order (Tables 3-5). These do not denote as high of a probability for a match as for a genus, but it is possible that those sequences could come from the same organism. This means it is also possible that those cultures can be directly connected back to those sampling times through the community's 16S rDNA found in the mass sequencing efforts. No isolates matched sequences that had been characterized to the species level. However, only 11 species were identified through the 16S rDNA communities so this likely contributed to the inability to match isolates to their corresponding species within the community profiles. In total, 46 of the 69 cultures were plausibly found to match a sequence within their associated 16S rDNA community profile.

Table 6a. Identification of general microbiology lab isolates as well as the determination of whether matching organisms were found in the corresponding 16S rDNA community.

Original Sample ID	Culture ID	Genus	Species	Percent DNA Match
D1S1	**15B-3**	*Arthrobacter*	*nicotinovorans*	99.1%
D1S1	**16A-2**	*Microbacteriaceae*	*bacterium*	100.0%
D1S1	**16A-2**	*Staphylococcus*	*saprophyticus*	99.6%
D1S2	**07A-4**	*Arthrobacter*	*nitroguajolicus*	100.0%
D1S2	**07B-3**	*Arthrobacter*	*nitroguajolicus*	98.0%
D1S2	**17A-1**	*Micrococcus*	*aloeverae*	100.0%
D1S2	**17A-2**	*Bacillus* [o]	*cereus*	99.6%
D1S2	**17B-1**	*Bacillus* [o]	*cereus*	100.0%
D1S2	**17B-3**	*Arthrobacter*	*nitroguajolicus*	98.0%
D1S3	**14A-3**	*Proteus* [g]	*vulgaris*	97.6%
D1S3	**14B-1**	*Proteus* [g]	*vulgaris*	97.1%
D1S3	**18A-2**	*Proteus* [g]	*vulgaris*	97.0%
D1S3	**18B-1**	*Morganella* [g]	*morganii*	98.4%
D1S4	**19A-1**	*Morganella* [g]	*morganii*	98.4%
D1S4	**19B-1**	*Bacillus* [o]	*cereus*	99.9%
D1S4	**19B-1**	*Proteus* [g]	*vulgaris*	97.0%
D1S5	**20A-3**	*Macrococcus*	*caeolyticus*	97.6%
D1S5	**20C-1**	*Dermacoccus*	*nishinomiyaensis*	96.9%
D1S6	**21A-1**	*Bacillus* [o]	*cereus*	100.0%
D1S6	**21A-2**	*Proteus* [g]	*vulgaris*	97.5%
D1S6	**21B-3**	*Myroides* [g]	*profundi*	91.5%
D1S7	**22A-2**	*Bacillus* [o]	*cereus*	100.0%
D1S7	**22B-1**	*Acinetobacter* [g]	*guillouiae*	88.4%
D2S1	**01A-2**	*Pseudomonas* [g]	*koreensis*	98.0%
D2S1	**01B-2**	*Nocardia*	*coeliaca*	100.0%
D2S1	**02A-1**	*Paenarthrobacter*	*nicotinovorans*	99.0%
D2S1	**02A-2**	*Pseudomonas* [g]	*koreensis*	97.9%

[g, o] denotes isolates that were matched to sequences in their sample's corresponding 16S rDNA community profile. Unmarked genera were not matched to any possible sequences for that sample's 16S rDNA community profile. No species were matched to the community profile as most sequences were only capable of being classified to the genus level.
[o] Matched sequences that were only classified to order
[g] Matched sequences that were only classified to genus

Table 6b. Identification of general microbiology lab isolates as well as the determination of whether matching organisms were found in the corresponding 16S rDNA community (cont.).

Original Sample ID	Culture ID	Genus	Species	Percent DNA Match
D2S1	**02B-2**	*Pseudomonas* [g]	*moraviensis*	97.9%
D2S2	**03A-1**	*Serratia* [f]	*liquefaciens*	97.1%
D2S2	**03B-2**	*Hafnia* [f]	*paralvei*	98.2%
D2S3	**04A-2**	*Staphylococcus* [o]	*sciuri*	100.0%
D2S3	**04B-2**	*Providencia* [g]	*alcalifaciens*	99.5%
D2S4	**05A-2**	*Bacillus* [o]	*mycoides*	100.0%
D2S4	**05B-2**	*Staphylococcus* [g]	*xylosus*	98.7%
D2S5	**06A-1**	*Bacillus*	*simplex or muralis*	98.4%
D2S5	**06A-3**	*Staphylococcus* [g]	*xylosus*	98.6%
D2S5	**06B-1**	*Bacillus* [o]	*cereus*	99.9%
D3S1	**08A-1**	*Lactobacillus* [g]	*paracasei*	99.6%
D3S1	**08A-2**	*Enhydrobacter*	*aerosaccus*	96.0%
D3S1	**08B-2**	*Massilia*	sp. WG5	96.2%
D3S1	**09A-3**	*Enterococcus*	*faecalis*	97.3%
D3S1	**09B-2**	*Corynebacterium* [g]	*striatum*	96.9%
D3S1	**09C-1**	*Dermacoccus*	*nishinomiyaensis*	98.7%
D3S1	**09C-2**	*Lactobacillus* [g]	*pentosus*	99.5%
D3S2	**10A-1**	*Staphylococcus* [g]	*surius*	98.6%
D3S2	**10B-1**	*Morganella* [g]	*morganii*	96.8%
D3S2	**10B-2**	*Kurthia* [g]	*zopfii*	99.6%
D3S3	**11A-3**	*Providencia* [g]	*vermicola*	96.1%
D3S3	**11B-2**	*Providencia* [g]	*rustigianii*	97.8%
D3S4	**12A-2**	*Lysinibacillus* [g]	*fusiforms*	99.8%
D3S4	**12B-2**	*Acinetobacter* [g]	*baumanii*	90.6%
D3S5	**13A-3**	*Comamonas* [g]	*terrigena*	95.2%
D3S5	**13B-2**	*Raoultella* [f]	*terrigena*	97.6%

[g, f, o] denotes isolatesthat were matched to sequences in their sample's corresponding 16S rDNA community profile. Unmarked genera were not matched to any possible sequences for that sample's 16S rDNA community profile. No species were matched to the community profile as most sequences were only capable of being classified to the genus level.
[o] Matched sequences that were only classified to order
[f] Matched sequences that were only classified to family
[g] Matched sequences that were only classified to genus

Table 7. Identification of senior research lab isolates as well as the determination of whether matching organisms were found in the corresponding 16S rDNA community.

Original Sample ID	Culture ID	Media	Genus	Species	Percent DNA Match
D1S1	WCU-101	DNB	*Microbacterium*	*pumilum*	95.1%
D1S4	WCU-114	DNB	*Arthrobacter*	*nitroguajolicus*	99.0%
D1S6	WCU-109	DNB	*Pseudomonas* [g]	*deceptionensis*	96.1%
D1S6	WCU-110	DR2A	*Pseudomonas* [g]	*lundensis*	96.0%
D2S2	WCU-106	DR2A	*Pseudomonas*	*moraviensis*	98.0%
D2S3	WCU-113	DR2A	*Providencia* [g]	*rustigianii*	97.9%
D2S3	WCU-115	DBHI	*Providencia* [g]	*vermicola*	97.1%
D2S3	WCU-116	DR2A	*Proteus* [g]	*vulgaris*	95.6%
D3S1	WCU-112	DNB	*Curtobacterium*	*citreum*	99.5%
D3S3	WCU-119	DR2A	*Providencia* [g]	*vermicola*	96.4%
D3S3	WCU-120	DR2A	*Kocuria*	*rhizophila*	95.6%
D3S3	WCU-121	DR2A	*Corynebacterium* [g]	*hansenii*	94.1%
D3S3	WCU-122	DR2A	*Sphingomonas*	*xinjiangensis*	91.9%
D3S5	WCU-103	DNB	*Flavobacterium* [g]	*piscis*	96.1%
D3S5	WCU-104	DNB	*Stenotrophomonas* [g]	*maltophilia*	97.5%
D3S5	WCU-108	DNB	*Janthinobacterium* [o]	*lividum*	99.4%

[g, o] denotes isolatesthat were matched to sequences in their sample's corresponding 16S rDNA community profile. Unmarked genera were not matched to any possible sequences for that sample's 16S rDNA community profile. No species were matched to the community profile as most sequences were only capable of being classified to the genus level.
[o] Matched sequences that were only classified to order
[g] Matched sequences that were only classified to genus

CHAPTER FOUR: DISCUSSION

Discussion of Observed Taxa within 16S rDNA Community Profiles

The presence of Actinobacteria early on in the decomposition process is not uncommon as there are five genera of Actinobacteria that are commonly found within the human microbiome: *Actinomyces, Bifidobacterium, Corynebacterium, Propionibacterium,* and *Rothia*.[28] *Bifidobacterium* and *Propionibacterium* were found in trace concentrations within some samples, which is likely due to *Bifidobacterium* primarily colonizing the gastrointestinal tract and *Propionibacterium* localizing within sebaceous follicles of the skin.[28] However, the other three genera were found in much larger quantities in this study (Figure 8). *Rothia* was only found in ADD 0 and 49 for Donor 1, but for ADD 0 it comprised the majority of the community's profile. The representative species, *R. dentocariosa,* produces lactate from glucose fermentation, which is the main carbon source for fermentation performed by *Veillonella* spp.[28] Interestingly, this latter species was found at ADD 0 for Donor 3 along with another lactate producer, *Streptococcus*.[28] *Actinomyces*, which contains species that metabolize the sialic acid in saliva, was found in the first four ADD profiles.[28] *Corynebacterium* were found in trace amounts throughout many of the samples, but was most heavily present in ADD 41 of Donor 2 and ADD 291 of Donor 3. These results are similar to those of other studies.[11,15] However, Adserias et al. found that as time progresses, the bacterial communities shift to becoming more like that of the soil related microbiota.[15] This includes the later *Corynebacterium* they found in their study.[15] The *Corynebacterium* species found at ADD 291 was unable to be characterized down to the species so it would be difficult to tell whether it belongs to the soil or human associated community.

Other noteworthy genera in this study also followed the trend of the microbiome becoming a more heavily soil-related community. *Lactobacillus* is only present in the first three sampling times and is most prevalent in the first sampling times for Donors 1 and 3 (ADD 0), which is unsurprising given that *Lactobacillus* spp. are part of the normal human oral microbiota.[55] Can et al., also described *Lactobacillus* as a genus that is only present in PMIs that were under 66 hours (assuming 27.5°C, this is approximately 76 ADD).[24] Similarly, *Granulicatella* species are also a natural inhabitant of the oral cavity and are only present in Donor 3 at ADD 0.[55,56] On the other end of the spectrum, within ADD 392, *Flavobacterium* and *Undibacterium* were present. These genera are both natural inhabitants of soil and water.[57,58] Notably, the abundance of fly associated taxa, including *Ignatzschineria*, *Vagococcus*, and *Wohlfahrtiimonas* were associated with the prevalence of fly activity throughout decomposition.[15]

Beginning at ADD 138, Family XI of order Clostridiales appears and persists throughout the end of sampling including the genera *Helcococcus*, *Peptoniphilus*, and *Tissierella*. All of these genera are either facultatively or obligately anaerobic.[59–61] This same family appeared in the study by Adserias et al. at the same time, which was from the end of the bloat stage, and persisted into advanced decay.[15] These genera are not considered to be part of the standard human oral microbiome as most of the human associated *Clostridia* are part of the families Lachnospiraceae, Peptostreptococcaceae, and Veillonellaceae.[15,55] Unlike the study by Adserias et al., the bloat stage was missed during this study. This may explain why the signature phylum of the bloat stage, the Tenericutes, was not found.[15] Tenericutes are members of the intestinal microbiota and require a host, possibly due to their lack of peptidoglycan-based cell walls.[15,62] The presence of obligate anaerobes suggests that at some point, an anoxic environment must

have been present for these microbes to exist in such large quantities. However, the creation of an anoxic environment has only been studied in the soil of the "cadaver decomposition island" (CDI) or the hot spot of nutrients surrounding decomposing animal remains.[19] In the study by Cobaugh et al., these anoxic environments created by a spike in bacterial activity promoted the growth of Clostridiales genera such as *Tissierella* and *Anaerosphaera*.[19] It also possible that sequences from this phylum, the Firmicutes, continue to be found in samples due to the ability of most, if not all, species within this phylum to form endospores.

The findings of this study do match those of others that have experienced the "Postmortem Clostridium Effect" (PCE).[23] The PCE centers only on the genus *Clostridium* and states that this genus is not only ubiquitous throughout decomposition, but also comprises the majority of the taxa that are present.[23] However, in the study that examined this effect, samples were only taken from spleen and liver tissue.[23] Based on the results of this study and the study performed by Adserias et al., the PCE is presumably not applicable to the human mouth.[15]

Discussion of the Relationship between 16S rDNA Community Profiles and PMI

When comparing the community compositions between samples at higher taxonomic levels, it appears as though there is no discernible difference between certain ADD ranges except for the distinction between samples with an ADD range of 0-50 and all other sampling events (Figures 9-11). This suggests that the thanatomicrobiome of the oral cavity, while easily accessible, may not be as predictive of PMI as other tissues or organs. The principal components analysis also shows a trend that it is not obvious from the heatmaps. The community profiles for samples from Donor 1 tend to be more consistent throughout decomposition (Figures 9-11). It is possible that this is due to the lower temperatures experienced by Donor 1 (Figure 1). Donor 1 experienced temperatures that were often 10°C, and at times even 30°C, lower than Donor 2 and

3 (Figure 1). This effect of temperature on the bacterial communities can likely be attributed to how temperature influences enzyme activity. Enzymes have 10° temperature quotients or Q_{10} values, which represent how the enzyme's activity changes in response to a 10°C change in temperature.[63] For some enzymes, changing the temperature by 10°C can double or even triple their activity.[63] This can drastically change an organism's rate of metabolism. While the effects of temperature differences should have been accounted for by using accumulated degree days, these results suggest that ADD alone may not be capable of standardizing the measurement of decomposition between cadavers exposed to vastly different temperatures. It is possible that incorporating how temperature can influence the metabolic rates of prevalent bacterial taxa could assist in creating a more accurate method of standardizing decomposition measurements.

The lack of clear relationships between bacterial communities and PMI could also be attributed to the low statistical power that this study had due to its small sample size. Unfortunately, due to the paucity of resources required to perform studies such as this and the costly DNA analysis that accompanies it, small sample sizes are not uncommon in thanatomicrobiome studies.[64]

Discussion of Bacterial Isolates

In total, 67% of all cultures isolated in the lab were capable of being linked to their respective 16S rDNA communities, at least at one taxonomic level. It is possible that the fact that not all cultures could be matched to the 16S rDNA sequence counterparts could be explained by one of two possibilities. 1) Given that cultures were obtained from different swabs than those used for DNA extraction, it is possible that the 16S rDNA communities did not represent every organism present at that sampling time. 2) Some cultures could be lab contaminants introduced by worker error. Either of these scenarios could also explain why there were some cases in which

cultured isolates matched genera found in the 16S rDNA communities of other samples, but not those found within their corresponding sampling time. The 67% match rate coupled with the wide metabolic diversity experienced in the isolates is promising for being able to match cultures with their role in the decomposition process once the shotgun metagenomics and metatranscriptomics data is analyzed. Ideally, the 16S rDNA sequence of each bacterial isolate will be matched to a corresponding sequence within the raw sequence data of the 16S rDNA community from the same sample time and donor. Finding a match would confirm the presence of that particular isolate within that specific community profile. In this way, links could be made between isolates and their role within that community during decomposition.

Future Work

Currently, the analysis of the metagenomic and metatranscriptomic work for this study is ongoing. Both datasets will be analyzed using the Microbial Genomics Module 4.1 (MGM) within the CLC Genomic Workbench 12.0 software (QIAGEN).[65] Once the tools within the module have been used to assemble a trimmed contig that has been searched for probable bacterial genes and coding DNA sequences (CDS), the CDS for each sample will be run through five databases in order to build functional profiles for each community. These databases will include the Protein Family (Pfam-A v32), Gene Ontology (GO), UniProt Reference Clusters 50 (UniRef50), SWISS-PROT, and Clusters of Orthologous Genes (COG) databases. Table 6 shows the number of raw shotgun metagenomic sequences received for each sample as well as the percent of sequences remaining after human DNA has been removed. It is anticipated that some of the isolates will be able to be matched directly to the metagenomic and metatranscriptomic sequences, which will tie them directly to decomposition processes and allow for further testing of the cultures for their roles in decomposition.

Table 8. Raw sequences for the shotgun metagenomics of each sample. Highlighted samples show which samples have suffered a significant loss in sequences due to the removal of human DNA.

Sample	Raw Sequence Count	Sequence Count After Trimming	Final Bacterial Sequence Count	Percent of Original
D1S1	6948738	6773515	277957	4%
D1S2	1774920	1765024	150809	8%
D1S3	5740498	5611643	5336292	93%
D1S4	5597330	5506972	5502429	98%
D1S5	7620987	7430921	7423941	97%
D1S6	5110985	4994394	4994331	98%
D1S7	7284681	7111835	7111702	98%
D2S1	5827053	5753670	1201912	21%
D2S2	3006265	2951267	2941322	98%
D2S3	4549152	4463614	4463488	98%
D2S4	5733028	5580928	5555481	97%
D2S5	6125143	6002814	5972044	98%
D3S1	4100787	4013669	1561053	38%
D3S2	6014653	5874385	5862022	97%
D3S3	6775805	6595188	6584581	97%
D3S4	2618001	2565603	2558819	98%
D3S5	5577125	5414862	5414064	97%

Conclusions

This study does exhibit similar results to those of other studies on the oral thanatomicrobiome, including those that have shown the oral microbiome to host a distinct microbiome from those of other decomposing organs and tissues. However, due to how easily the environment can influence the oral microbiota, it appears as though sampling of the oral cavity would need to be further evaluated for use in a forensic setting. Although a relationship

between community structure and decomposition state was not discerned from the 16S rDNA community profiles, future work on the fluctuations in community function could uncover a connection between functional changes and decomposition.

Due to how easily the mouth can be accessed, it would be a valuable sampling site for both researchers and forensic scientists alike if a relationship can be found between the oral microbiome and the process of decomposition. However, this study also shows the necessity for larger scale studies with more cadavers for higher power statistical analyses across wider time frames and seasons to account for the disparate temperatures and precipitation that can be experienced within only a two-month time frame. Also, this study demonstrates the need for a more accurate method of standardizing decomposition measurements. Finding a method that would account not only for the cumulative effect of temperature, but also the effects of precipitation. It appears that a measurement such as this would prove useful in the development of more accurate predictive models based on bacterial communities. The combination of more, large-scale studies as well as a method for dating decomposition that accounts for multiple factors, still has the potential to uncover a relationship between the post-mortem microbiome of the mouth and human decomposition and it may be the case that this relationship lies with the functional shifts exhibited by the community as decomposition progresses.

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APPENDIX A: DILUTE MEDIA RECIPES

Table A1. Recipe for dilute (1%) Reasoner's 2 agar (DR2A).

Ingredient	Amount (g/L)
Proteose Peptone	0.05
Casamino Acids	0.05
Yeast Extract	0.05
Dextrose	0.05
Soluble Starch	0.05
Dipotassium Phosphate	0.03
Magnesium Sulfate per $7H_20$	0.005
Sodium Pyruvate	0.03
Agar	20

Table A2. Recipe for dilute (1%) brain heart infusion agar (DBHI).

Ingredient	Amount (g/L)
Brain/Heart Infusion from Solids	0.08
Peptic Digest of Animal Tissue	0.05
Pancreatic Digest of Casein	0.16
Sodium Chloride	0.05
Glucose	0.02
Disodium Hydrogen Phosphate	0.025
Agar	20

Table A3. Recipe for dilute (10%) nutrient broth agar (DNB).

Ingredient	Amount (g/L)
Beef Extract	0.3
Peptone	0.5
Agar	20

APPENDIX B: RAW OTU COUNTS FOR EACH TAXONOMIC LEVEL

OTU Counts by Phylum

Table B1. Raw OTU counts from QIIME2 for each 16S rDNA community sampled from three human donors over multiple sampling times throughout decomposition (D: donor; S: sample time). OTU counts are shown at the phylum level.

Sample ID	Actinobacteria	Bacteroidetes	Epsilonbacteraeota	Firmicutes	Fusobacteria	Proteobacteria	Verrucomicrobia	Total OTU
D1S1	2078	0	0	903	0	0	0	2981
D1S2	747	261	0	2645	0	1434	179	5266
D1S3	11	2	0	7964	0	617	0	8594
D1S4	0	5	0	3343	0	574	0	3922
D1S5	0	0	0	6215	0	369	0	6584
D1S6	0	10	0	1914	0	2337	0	4261
D1S7	8	66	0	2935	0	3129	0	6138
D2S1	639	755	15	1961	65	1458	0	4893
D2S2	23	0	0	475	0	4551	0	5049
D2S3	0	113	0	2875	0	610	0	3598
D2S4	0	5	0	4054	0	507	0	4566
D2S5	7	2	0	2653	0	97	0	2759
D3S1	1387	187	87	3823	0	0	0	5484
D3S2	25	436	0	1984	0	6005	0	8450
D3S3	182	6	0	3713	0	197	0	4098
D3S4	2457	7	0	435	0	1195	0	4094
D3S5	50	297	16	8	0	858	0	1229

OTU Counts by Class

Table B2. Raw OTU counts from QIIME2 for each 16S rDNA community sampled from three human donors over multiple sampling times throughout decomposition (D: donor; S: sample time). OTU counts are shown at the class level.

Sample ID	Actinobacteria	Bacteroidia	Campylobacteria	Bacilli	Clostridia	Erysipelotrichia	Negativicutes	Unspecified Firmicutes (p)	Fusobacteriia	Alphaproteobacteria	Deltaproteobacteria	Gammaproteobacteria	Verrucomicrobiae	Total OTU
D1S1	2078	0	0	903	0	0	0	0	0	0	0	0	0	2981
D1S2	747	261	0	2558	87	0	0	0	0	0	0	1434	179	5266
D1S3	11	2	0	7956	8	0	0	0	0	0	0	617	0	8594
D1S4	0	5	0	2233	1090	20	0	0	0	0	0	574	0	3922
D1S5	0	0	0	5975	231	9	0	0	0	0	0	369	0	6584
D1S6	0	10	0	1887	25	2	0	0	0	0	0	2337	0	4261
D1S7	8	66	0	2915	17	3	0	0	0	5	0	3124	0	6138
D2S1	639	755	15	859	1051	0	51	0	65	0	0	1458	0	4893
D2S2	23	0	0	340	135	0	0	0	0	0	0	4551	0	5049
D2S3	0	113	0	698	2124	44	0	9	0	0	0	610	0	3598
D2S4	0	5	0	3193	837	24	0	0	0	0	0	507	0	4566
D2S5	7	2	0	1923	723	5	0	2	0	0	0	97	0	2759
D3S1	1387	187	87	3514	0	0	309	0	0	0	0	0	0	5484
D3S2	25	436	0	1917	67	0	0	0	0	0	0	6005	0	8450
D3S3	182	6	0	2276	1428	9	0	0	0	0	0	197	0	4098
D3S4	2457	7	0	369	66	0	0	0	0	0	0	1195	0	4094
D3S5	50	297	16	2	6	0	0	0	0	28	117	713	0	1229

(p): name of phylum

OTU Counts by Order

Table B3. Raw OTU counts from QIIME2 for each 16S rDNA community sampled from three human donors over multiple sampling times throughout decomposition (D: donor; S: sample time). OTU counts are shown for orders within the Actinobacteria, Bacteroidetes, Firmicutes, Fusobacteria, and Verrucomicrobia phyla.

Sample ID	Actinomycetales	Bifidobacteriales	Corynebacteriales	Micrococcales	Propionibacteriales	Bacteroidales	Chitinophagales	Flavobacteriales	Sphingobacteriales	Bacillales	Lactobacillales	Unspecified Bacilli (c)	Clostridiales	Unspecified Clostridia (c)	Erysipelotrichales	Selenomonadales	Unspecified Firmicutes (p)	Fusobacteriales	Verrucomicrobiales	Total OTU
D1S1	0	0	0	2078	0	0	0	0	0	0	903	0	0	0	0	0	0	0	0	2981
D1S2	0	0	15	716	16	261	0	0	0	212	2346	0	87	0	0	0	0	0	179	5266
D1S3	0	0	0	11	0	2	0	0	0	7511	445	0	8	0	0	0	0	0	0	8594
D1S4	0	0	0	0	0	5	0	0	0	1686	547	0	1090	0	20	0	0	0	0	3922
D1S5	0	0	0	0	0	0	0	0	0	5845	130	0	231	0	9	0	0	0	0	6584
D1S6	0	0	0	0	0	2	0	8	0	1784	103	0	25	0	2	0	0	0	0	4261
D1S7	0	0	0	8	0	0	0	66	0	2610	305	0	17	0	3	0	0	0	0	6138
D2S1	176	0	294	167	2	739	0	16	0	244	615	0	1051	0	0	51	0	65	0	4893
D2S2	17	0	0	0	6	0	0	0	0	82	258	0	135	0	0	0	0	0	0	5049
D2S3	0	0	0	0	0	113	0	0	0	467	231	0	2114	10	44	0	9	0	0	3598
D2S4	0	0	0	0	0	5	0	0	0	2645	543	5	835	2	24	0	0	0	0	4566
D2S5	0	0	7	0	0	0	0	2	0	1637	286	0	723	0	5	0	2	0	0	2759
D3S1	972	148	18	249	0	187	0	0	0	23	3491	0	0	0	0	309	0	0	0	5484
D3S2	0	0	14	11	0	35	0	401	0	1483	434	0	67	0	0	0	0	0	0	8450
D3S3	0	0	174	8	0	6	0	0	0	1182	1094	0	1428	0	9	0	0	0	0	4098
D3S4	0	0	2457	0	0	5	0	0	2	237	132	0	66	0	0	0	0	0	0	4094
D3S5	0	0	32	13	5	18	26	220	33	0	2	0	6	0	0	0	0	0	0	1229

(p): name of phylum, (c): name of class

Table B4. Raw OTU counts from QIIME2 for each 16S rDNA community sampled from three human donors over multiple sampling times throughout decomposition (D: donor; S: sample time). OTU counts are shown for orders within the Proteobacteria phylum.

Sample ID	Campylobacterales	Micavibrionales	Rhizobiales	Bdellovibrionales	Oligoflexales	Betaproteobacteriales	Cardiobacteriales	Enterobacteriales	Legionellales	Pasteurellales	Pseudomonadales	Xanthomonadales	Unspecified Gammaproteobacteria (c)	Total OTU
D1S1	0	0	0	0	0	0	0	0	0	0	0	0	0	2981
D1S2	0	0	0	0	0	0	1431	3	0	0	0	0	0	5266
D1S3	0	0	0	0	0	10	448	155	0	0	4	0	0	8594
D1S4	0	0	0	0	0	0	563	4	0	0	7	0	0	3922
D1S5	0	0	0	0	0	0	268	2	0	0	99	0	0	6584
D1S6	0	0	0	0	0	2	262	6	0	0	2065	0	2	4261
D1S7	0	0	5	0	0	44	1060	8	0	0	2009	0	3	6138
D2S1	15	0	0	0	0	409	0	0	0	467	582	0	0	4893
D2S2	0	0	0	0	0	0	4475	76	0	0	0	0	0	5049
D2S3	0	0	0	0	0	0	598	8	0	0	4	0	0	3598
D2S4	0	0	0	0	0	0	311	0	0	0	196	0	0	4566
D2S5	0	0	0	0	0	3	40	0	0	0	54	0	0	2759
D3S1	87	0	0	0	0	0	0	0	0	0	0	0	0	5484
D3S2	0	0	0	0	0	32	750	17	0	0	5206	0	0	8450
D3S3	0	0	0	0	0	0	160	31	0	0	6	0	0	4098
D3S4	0	0	0	0	0	30	1038	61	0	0	64	2	0	4094
D3S5	16	5	23	11	106	423	2	89	12	0	184	3	0	1229

(c): name of class

OTU Counts by Family

Table B5. Raw OTU counts from QIIME2 for each 16S rDNA community sampled from three human donors over multiple sampling times throughout decomposition (D: donor; S: sample time). OTU counts are shown for families within the Actinobacteria, Bacteroidetes, Fusobacteria, and Verrucomicrobia.

Sample ID	Actinomycetaceae	Bifidobacteriaceae	Corynebacteriaceae	Dietziaceae	Nocardiaceae	Microbacteriaceae	Micrococcaceae	Propionibacteriaceae	Bacteroidaceae	Dysgonomonadaceae	Porphyromonadaceae	Prevotellaceae	Rikenellaceae	Tannerellaceae	Chitinophagaceae	Unspecified Chitinophagales (o)	Flavobacteriaceae	Sphingobacteriaceae	Fusobacteriaceae	Leptotrichiaceae	Akkermansiaceae	Total OTU
D1S1	0	0	0	0	0	0	2078	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2981
D1S2	0	0	13	0	2	0	716	16	122	90	0	0	41	8	0	0	0	0	0	0	179	5266
D1S3	0	0	0	0	0	0	11	0	0	2	0	0	0	0	0	0	0	0	0	0	0	8594
D1S4	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	3922
D1S5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6584
D1S6	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	8	0	0	0	0	4261
D1S7	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	66	0	0	0	0	6138
D2S1	176	0	294	0	0	0	167	2	0	0	176	563	0	0	0	0	16	0	41	24	0	4893
D2S2	17	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	5049
D2S3	0	0	0	0	0	0	0	0	113	0	0	0	0	0	0	0	0	0	0	0	0	3598
D2S4	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	4566
D2S5	0	0	0	5	2	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	2759
D3S1	972	148	18	0	0	0	249	0	0	0	0	187	0	0	0	0	0	0	0	0	0	5484
D3S2	0	0	11	3	0	4	7	0	35	0	0	0	0	0	0	0	401	0	0	0	0	8450
D3S3	0	0	165	2	7	3	5	0	6	0	0	0	0	0	0	0	0	0	0	0	0	4098
D3S4	0	0	2457	0	0	0	0	0	5	0	0	0	0	0	0	0	0	2	0	0	0	4094
D3S5	0	0	25	0	7	5	8	5	0	18	0	0	0	0	11	15	220	33	0	0	0	1229

(o): name of order

Table B6. Raw OTU counts from QIIME2 for each 16S rDNA community sampled from three human donors over multiple sampling times throughout decomposition (D: donor; S: sample time). OTU counts are shown for families within the Firmicutes phylum.

Sample ID	Bacillaceae	Family XI	Planococcaceae	Staphylococcaceae	Unspecified Bacillales (o)	Aerococcaceae	Carnobacteriaceae	Enterococcaceae	Lactobacillaceae	Leuconostocaceae	Streptococcaceae	Unspecified Lactobacillales (o)	Unspecified Bacilli (c)	Clostridiaceae 1	Family XI	Family XIII	Lachnospiraceae	Peptococcaceae	Peptostreptococcaceae	Unspecified Clostridiales (o)	Unspecified Clostridia (c)	Erysipelotrichaceae	Veillonellaceae	Unspecified Firmicutes (p)	Total OTU
D1S1	0	0	0	0	0	0	0	0	903	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2981
D1S2	0	0	10	198	4	0	2	2063	281	0	0	0	0	12	25	0	24	0	26	0	0	0	0	0	5266
D1S3	0	0	7509	2	0	0	3	442	0	0	0	0	0	0	0	0	0	0	8	0	0	0	0	0	8594
D1S4	0	0	1411	0	275	0	8	539	0	0	0	0	0	0	948	0	0	0	142	0	0	20	0	0	3922
D1S5	0	0	3178	0	2667	0	0	130	0	0	0	0	0	0	220	0	0	0	11	0	0	9	0	0	6584
D1S6	0	0	1420	0	364	0	3	100	0	0	0	0	0	0	25	0	0	0	0	0	0	2	0	0	4261
D1S7	0	0	2397	0	213	0	0	305	0	0	0	0	0	0	17	0	0	0	0	0	0	3	0	0	6138
D2S1	0	244	0	0	0	23	279	0	4	0	309	0	0	1019	8	5	19	0	0	0	0	0	51	0	4893
D2S2	7	0	60	0	15	0	0	248	0	5	5	0	0	10	0	0	0	0	125	0	0	0	0	0	5049
D2S3	0	0	145	0	322	0	0	231	0	0	0	0	0	94	1862	0	0	53	69	36	10	44	0	9	3598
D2S4	0	0	936	10	1699	0	0	536	7	0	0	0	5	41	733	0	0	4	32	25	2	24	0	0	4566
D2S5	0	0	426	9	1202	0	0	284	2	0	0	0	0	65	626	0	0	3	24	5	0	5	0	2	2759
D3S1	0	21	0	2	0	0	373	0	2324	0	794	0	0	0	0	0	0	0	0	0	0	0	309	0	5484
D3S2	5	0	1411	42	25	0	68	366	0	0	0	0	0	2	55	0	0	0	10	0	0	0	0	0	8450
D3S3	0	0	357	17	808	0	15	1066	0	0	0	13	0	28	1084	0	0	2	314	0	0	9	0	0	4098
D3S4	0	0	142	3	92	0	0	132	0	0	0	0	0	11	40	0	0	0	15	0	0	0	0	0	4094
D3S5	0	0	0	0	0	0	0	2	0	0	0	0	0	0	4	0	0	0	2	0	0	0	0	0	1229

(p): name of phylum, (c): name of class, (o): name of order

Table B7. Raw OTU counts from QIIME2 for each 16S rDNA community sampled from three human donors over multiple sampling times throughout decomposition (D: donor; S: sample time). OTU counts are shown for families within the Proteobacteria phylum.

Sample ID	Uncultured Micavibrionales (o)	Rhizobiaceae	Burkholderiaceae	Neisseriaceae	Rhodocyclaceae	Unspecified Betaproteobacteriales (o)	Bdellovibrionaceae	Arcobacteraceae	Campylobacteraceae	Wohlfahrtiimonadaceae	Enterobacteriaceae	Legionellaceae	Pasteurellaceae	Moraxellaceae	Pseudomonadaceae	Xanthomonadaceae	Unspecified Gammaproteobacteria (c)	Oligoflexaceae	Total OTU
D1S1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2981
D1S2	0	0	0	0	0	0	0	0	0	1431	3	0	0	0	0	0	0	0	5266
D1S3	0	0	10	0	0	0	0	0	0	448	155	0	0	0	4	0	0	0	8594
D1S4	0	0	0	0	0	0	0	0	0	563	4	0	0	0	7	0	0	0	3922
D1S5	0	0	0	0	0	0	0	0	0	268	2	0	0	0	99	0	0	0	6584
D1S6	0	0	2	0	0	0	0	0	0	262	6	0	0	21	2044	0	2	0	4261
D1S7	0	5	44	0	0	0	0	0	0	1060	8	0	0	126	1883	0	3	0	6138
D2S1	0	0	0	409	0	0	0	0	15	0	0	0	467	0	582	0	0	0	4893
D2S2	0	0	0	0	0	0	0	0	0	4475	76	0	0	0	0	0	0	0	5049
D2S3	0	0	0	0	0	0	0	0	0	598	8	0	0	2	2	0	0	0	3598
D2S4	0	0	0	0	0	0	0	0	0	311	0	0	0	0	196	0	0	0	4566
D2S5	0	0	3	0	0	0	0	0	0	40	0	0	0	0	54	0	0	0	2759
D3S1	0	0	0	0	0	0	0	0	87	0	0	0	0	0	0	0	0	0	5484
D3S2	0	0	24	8	0	0	0	0	0	750	17	0	0	5191	15	0	0	0	8450
D3S3	0	0	0	0	0	0	0	0	0	160	31	0	0	6	0	0	0	0	4098
D3S4	0	0	30	0	0	0	0	0	0	1038	61	0	0	49	15	2	0	0	4094
D3S5	5	23	270	0	10	143	11	16	0	2	89	12	0	55	129	3	0	106	1229

(c): name of class, (o): name of order

OTU Counts by Genus

Genera OTU Counts within the Actinobacteria, Bacteroidetes, Fusobacteria, and Verrucomicrobia Phyla

Table B8. Raw OTU counts from QIIME2 for each 16S rDNA community sampled from three human donors over multiple sampling times throughout decomposition (D: donor; S: sample time). OTU counts are shown for genera within the Actinobacteria phylum.

Sample ID	*Actinomyces*	*Alloscardovia*	*Bifidobacterium*	*Corynebacterium*	*Corynebacterium 1*	*Dietzia*	*Rhodococcus*	*Leucobacter*	*Microbacterium*	*Arthrobacter*	*Glutamicibacter*	*Paenarthrobacter*	*Pseudarthrobacter*	*Rothia*	*Acidipropionibacterium*	*Cutibacterium*	*Pseudopropionibacterium*	Total OTU
D1S1	0	0	0	0	0	0	0	0	0	0	0	0	0	2078	0	0	0	2981
D1S2	0	0	0	0	13	0	2	0	0	0	0	37	9	670	0	16	0	5266
D1S3	0	0	0	0	0	0	0	0	0	0	0	9	2	0	0	0	0	8594
D1S4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3922
D1S5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6584
D1S6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4261
D1S7	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0	6138
D2S1	176	0	0	294	0	0	0	0	0	0	0	0	0	167	0	0	2	4893
D2S2	17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	5049
D2S3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3598
D2S4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4566
D2S5	0	0	0	0	0	5	2	0	0	0	0	0	0	0	0	0	0	2759
D3S1	972	31	117	11	7	0	0	0	0	0	0	0	0	249	0	0	0	5484
D3S2	0	0	0	0	11	3	0	4	0	0	7	0	0	0	0	0	0	8450
D3S3	0	0	0	0	165	2	7	3	0	3	2	0	0	0	0	0	0	4098
D3S4	0	0	0	0	2457	0	0	0	0	0	0	0	0	0	0	0	0	4094
D3S5	0	0	0	0	25	0	7	0	5	8	0	0	0	0	5	0	0	1229

Table B9. Raw OTU counts from QIIME2 for each 16S rDNA community sampled from three human donors over multiple sampling times throughout decomposition (D: donor; S: sample time). OTU counts are shown for genera within the Bacteroidetes, Fusobacteria, and Verrucomicrobia phyla.

Sample ID	*Bacteroides*	*Dysgonomonas*	*Porphyromonas*	*Alloprevotella*	*Prevotella*	*Prevotella* 6	*Prevotella* 7	*Alistipes*	*Parabacteroides*	*Taibaiella*	Unspecified Chitinophagales (o)	*Capnocytophaga*	*Flavobacterium*	*Myroides*	*Pedobacter*	*Sphingobacterium*	*Fusobacterium*	*Leptotrichia*	*Akkermansia*	Total OTU
D1S1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2981
D1S2	122	90	0	0	0	0	0	41	8	0	0	0	0	0	0	0	0	0	179	5266
D1S3	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8594
D1S4	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3922
D1S5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6584
D1S6	2	0	0	0	0	0	0	0	0	0	0	0	4	4	0	0	0	0	0	4261
D1S7	0	0	0	0	0	0	0	0	0	0	0	0	12	54	0	0	0	0	0	6138
D2S1	0	0	176	65	64	0	434	0	0	0	0	16	0	0	0	0	41	24	0	4893
D2S2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5049
D2S3	113	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3598
D2S4	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4566
D2S5	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	2759
D3S1	0	0	0	0	0	169	18	0	0	0	0	0	0	0	0	0	0	0	0	5484
D3S2	35	0	0	0	0	0	0	0	0	0	0	0	4	397	0	0	0	0	0	8450
D3S3	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4098
D3S4	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	4094
D3S5	0	18	0	0	0	0	0	0	0	11	15	0	220	0	8	25	0	0	0	1229

(o): name of order

Genera OTU Counts within the Firmicutes Phylum

Table B10. Raw OTU counts from QIIME2 for each 16S rDNA community sampled from three human donors over multiple sampling times throughout decomposition (D: donor; S: sample time). OTU counts are shown for genera within the Bacilli class of the Firmicutes phylum.

Sample ID	*Bacillus*	*Gemella*	*Kurthia*	*Lysinibacillus*	*Savagea*	*Sporosarcina*	Unspecified Planococcaceae (f)	*Macrococcus*	*Staphylococcus*	Unspecified Staphylococcaceae (f)	Unspecified Bacillales (o)	*Abiotrophia*	*Carnobacterium*	*Granulicatella*	*Enterococcus*	*Vagococcus*	Unspecified Enterococcaceae (f)	*Lactobacillus*	Unspecified Lactobacillaceae (f)	*Leuconostoc*	*Streptococcus*	Unspecified Lactobacillales (o)	Unspecified Bacilli (c)	Total OTU
D1S1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	903	0	0	0	0	0	2981
D1S2	0	0	0	5	0	0	5	0	198	0	4	0	2	0	35	2028	0	281	0	0	0	0	0	5266
D1S3	0	0	0	7489	0	11	9	0	2	0	0	0	3	0	0	442	0	0	0	0	0	0	0	8594
D1S4	0	0	0	48	0	47	1316	0	0	0	275	0	8	0	414	125	0	0	0	0	0	0	0	3922
D1S5	0	0	0	34	5	651	2488	0	0	0	2667	0	0	0	68	62	0	0	0	0	0	0	0	6584
D1S6	0	0	0	168	0	831	421	0	0	0	364	0	3	0	7	93	0	0	0	0	0	0	0	4261
D1S7	0	0	0	587	0	1245	565	0	0	0	213	0	0	0	2	303	0	0	0	0	0	0	0	6138
D2S1	0	244	0	0	0	0	0	0	0	0	0	23	0	279	0	0	0	4	0	0	309	0	0	4893
D2S2	7	0	0	6	0	6	48	0	0	0	15	0	0	0	0	248	0	0	0	5	5	0	0	5049
D2S3	0	0	0	0	0	5	140	0	0	0	322	0	0	0	148	83	0	0	0	0	0	0	0	3598
D2S4	0	0	0	12	0	37	887	0	10	0	1699	0	0	0	229	297	10	3	4	0	0	0	5	4566
D2S5	0	0	2	15	0	36	373	0	9	0	1202	0	0	0	183	92	9	0	2	0	0	0	0	2759
D3S1	0	21	0	0	0	0	0	0	2	0	0	0	0	373	0	0	0	2324	0	0	794	0	0	5484
D3S2	5	0	705	383	0	90	233	0	42	0	25	0	68	0	2	364	0	0	0	0	0	0	0	8450
D3S3	0	0	15	53	0	9	280	6	2	9	808	0	15	0	459	607	0	0	0	0	0	13	0	4098
D3S4	0	0	0	3	0	13	126	0	0	3	92	0	0	0	62	70	0	0	0	0	0	0	0	4094
D3S5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	1229

(c): name of class, (o): name of order, (f): name of family

Table B11. Raw OTU counts from QIIME2 for each 16S rDNA community sampled from three human donors over multiple sampling times throughout decomposition (D: donor; S: sample time). OTU counts are shown for genera within the Clostridia, Erysipelotrichia, and Negativicutes classes of the Firmicutes phylum.

Sample ID	*Clostridium sensu stricto* 1	*Clostridium sensu stricto* 7	*Hathewaya*	*Proteiniclasticum*	Unspecified Clostridiaceae 1 (f)	*Gallicola*	*Helcococcus*	*Parvimonas*	*Peptoniphilus*	*Tissierella*	Family XI W5053 (g)	Unspecified Family XI (f)	*Eubacterium nodatum* group	*Hungatella*	*Stomatobaculum*	*Cryptanaerobacter*	*Paraclostridium*	*Peptostreptococcus*	Unspecified Peptostreptococcaceae (f)	Unspecified Clostridiales (o)	Unspecified Clostridia (c)	*Erysipelothrix*	*Megasphaera*	*Veillonella*	Unspecified Firmicutes (p)	Total OTU
D1S1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2981
D1S2	5	4	0	3	0	0	0	0	25	0	0	0	0	24	0	0	26	0	0	0	0	0	0	0	0	5266
D1S3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0	8594
D1S4	0	0	0	0	0	0	437	0	0	504	0	7	0	0	0	0	13	129	0	0	0	20	0	0	0	3922
D1S5	0	0	0	0	0	2	22	0	0	181	2	13	0	0	0	0	0	11	0	0	0	9	0	0	0	6584
D1S6	0	0	0	0	0	0	9	0	0	16	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	4261
D1S7	0	0	0	0	0	0	8	0	0	9	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	6138
D2S1	824	0	195	0	0	0	0	8	0	0	0	0	5	0	19	0	0	0	0	0	0	0	7	44	0	4893
D2S2	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	113	0	12	0	0	0	0	0	0	5049
D2S3	17	14	63	0	0	23	559	0	46	1137	26	71	0	0	0	53	24	41	4	36	10	44	0	0	9	3598
D2S4	3	11	27	0	0	12	273	0	48	347	30	23	0	0	0	4	7	25	0	25	2	24	0	0	0	4566
D2S5	0	28	37	0	0	7	376	0	58	151	26	8	0	0	0	3	1	20	3	5	0	5	0	0	2	2759
D3S1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	309	0	5484
D3S2	0	0	0	2	0	9	27	0	10	7	2	0	0	0	0	0	0	10	0	0	0	0	0	0	0	8450
D3S3	2	24	0	0	2	55	462	0	364	160	38	5	0	0	0	2	0	314	0	0	0	9	0	0	0	4098
D3S4	3	2	3	0	3	2	8	0	24	0	6	0	0	0	0	0	0	15	0	0	0	0	0	0	0	4094
D3S5	0	0	0	0	0	0	2	0	2	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	1229

(p): name of phylum, (c): name of class, (o): name of order, (f): name of family, (g): name of genus

Genera OTU Counts within the Proteobacteria Phylum

Table B12. Raw OTU counts from QIIME2 for each 16S rDNA community sampled from three human donors over multiple sampling times throughout decomposition (D: donor; S: sample time). OTU counts are shown for genera within the Alphaproteobacteria, Betaproteobacteria, Deltaproteobacteria, Epsilonproteobacteria, and Oligoflexia classes of the Proteobacteria phylum.

Sample ID	Unspecified Micavibrionales (o)	*Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*	*Pseudochrobactrum*	*Acidovorax*	*Aquabacterium*	*Comamonas*	*Paenalcaligenes*	*Undibacterium*	Unspecified Burkholderiaceae (f)	*Neisseria*	*Vitreoscilla*	*Azospira*	Unspecified Betaproteobacteriales (o)	*Bdellovibrio*	*Arcobacter*	*Campylobacter*	*Oligoflexus*	Total OTU
D1S1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2981
D1S2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5266
D1S3	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	8594
D1S4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3922
D1S5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6584
D1S6	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	4261
D1S7	0	0	5	0	0	0	44	0	0	0	0	0	0	0	0	0	0	6138
D2S1	0	0	0	0	0	0	0	0	0	409	0	0	0	0	0	15	0	4893
D2S2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5049
D2S3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3598
D2S4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4566
D2S5	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	2759
D3S1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	87	0	5484
D3S2	0	0	0	0	0	24	0	0	0	0	8	0	0	0	0	0	0	8450
D3S3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4098
D3S4	0	0	0	0	0	30	0	0	0	0	0	0	0	0	0	0	0	4094
D3S5	5	14	9	13	61	29	0	160	7	0	0	10	143	11	16	0	106	1229

(o): name of order, (f): name of family

Table B13. Raw OTU counts from QIIME2 for each 16S rDNA community sampled from three human donors over multiple sampling times throughout decomposition (D: donor; S: sample time). OTU counts are shown for genera within the Gammaproteobacteria class of the Proteobacteria phylum.

Sample ID	***Ignatzschineria***	***Wohlfahrtiimonas***	***Morganella***	***Pantoea***	***Proteus***	***Providencia***	**Unspecified Enterobacteriaceae (f)**	***Legionella***	***Haemophilus***	***Acinetobacter***	***Alkanindiges***	***Pseudomonas***	**Unspecified Pseudomonadaceae (f)**	***Stenotrophomonas***	**Unspecified Gammaproteobacteria (c)**	**Total OTU**
D1S1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2981
D1S2	1431	0	0	0	3	0	0	0	0	0	0	0	0	0	0	5266
D1S3	429	19	86	0	26	43	0	0	0	0	0	4	0	0	0	8594
D1S4	550	13	2	0	2	0	0	0	0	0	0	7	0	0	0	3922
D1S5	268	0	0	0	2	0	0	0	0	0	0	99	0	0	0	6584
D1S6	262	0	0	0	4	2	0	0	0	21	0	2037	7	0	2	4261
D1S7	1057	3	3	0	3	2	0	0	0	126	0	1883	0	0	3	6138
D2S1	0	0	0	0	0	0	0	0	467	0	0	582	0	0	0	4893
D2S2	1901	2574	35	0	4	12	25	0	0	0	0	0	0	0	0	5049
D2S3	509	89	2	0	2	4	0	0	0	2	0	2	0	0	0	3598
D2S4	308	3	0	0	0	0	0	0	0	0	0	196	0	0	0	4566
D2S5	35	5	0	0	0	0	0	0	0	0	0	54	0	0	0	2759
D3S1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5484
D3S2	740	10	2	0	4	11	0	0	0	5191	0	15	0	0	0	8450
D3S3	160	0	0	0	9	19	3	0	0	6	0	0	0	0	0	4098
D3S4	1038	0	2	5	6	33	15	0	0	44	5	15	0	2	0	4094
D3S5	2	0	0	0	0	0	89	12	0	55	0	129	0	3	0	1229

(c): name of class, (f): name of family

OTU Counts by Species

Species OTU Counts within the Actinobacteria, Bacteroidetes, Fusobacteria, and Verrucomicrobia Phyla

Table B14. Raw OTU counts from QIIME2 for each 16S rDNA community sampled from three human donors over multiple sampling times throughout decomposition (D: donor; S: sample time). OTU counts are shown for species within the Actinobacteria phylum.

Sample ID	*Actinomyces* AT	*Actinomyces* UB	Unspecified *Actinomyces*	*Alloscardovia* AT	*Bifidobacterium longum* subsp. *longum*	*Corynebacterium* 1 AT	*Corynebacterium xerosis*	*Corynebacterium* 1 UB	*Corynebacterium* 1 UO	Unspecified *Corynebacterium* 1	*Corynebacterium* AT	*Corynebacterium* UB	Unspecified *Dietzia*	*Rhodococcus erythropolis*	*Leucobacter* AT	Unspecified *Leucobacter*	*Microbacterium* AT	*Arthrobacter* AT	*Glutamicibacter* AT	*Paenarthrobacter* AT	Unspecified *Pseudarthrobacter*	*Rothia* UB	*Acidipropionibacterium* AT	*Cutibacterium* UO	*Pseudopropionibacterium* UB	Total OTU
D1S1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2078	0	0	0	2981
D1S2	0	0	0	0	0	0	0	9	4	0	0	0	0	2	0	0	0	0	0	37	9	670	0	16	0	5266
D1S3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	2	0	0	0	0	8594
D1S4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3922
D1S5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6584
D1S6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4261
D1S7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	6138
D2S1	171	5	0	0	0	0	0	0	0	0	273	21	0	0	0	0	0	0	0	0	0	167	0	0	2	4893
D2S2	2	6	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	5049
D2S3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3598
D2S4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4566
D2S5	0	0	0	0	0	0	0	0	0	0	0	0	5	2	0	0	0	0	0	0	0	0	0	0	0	2759
D3S1	972	0	0	31	117	5	0	0	2	0	11	0	0	0	0	0	0	0	0	0	0	249	0	0	0	5484
D3S2	0	0	0	0	0	9	2	0	0	0	0	0	3	0	0	4	0	0	7	0	0	0	0	0	0	8450
D3S3	0	0	0	0	0	142	5	0	0	18	0	0	2	7	0	3	0	3	2	0	0	0	0	0	0	4098
D3S4	0	0	0	0	0	2453	2	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4094
D3S5	0	0	0	0	0	25	0	0	0	0	0	0	0	7	0	0	5	8	0	0	0	0	5	0	0	1229

AT = Ambiguous taxa, UB: uncultured bacterium, UO: uncultured organism

Table B15. Raw OTU counts from QIIME2 for each 16S rDNA community sampled from three human donors over multiple sampling times throughout decomposition (D: donor; S: sample time). OTU counts are shown for species within the Bacteroidetes, Fusobacteria, and Verrucomicrobia phyla.

Sample ID	*Bacteroides* AT	*Bacteroides* UO	*Bacteroides* uncultured prokaryote	Unspecified *Bacteroides*	*Dysgonomonas* AT	Unspecified *Dysgonomonas*	*Porphyromonas* UB	*Alloprevotella* UB	Unspecified *Alloprevotella*	*Prevotella* 6 UB	*Prevotella* 7 AT	*Prevotella* 7 UB	*Prevotella* UB	Unspecified *Prevotella*	*Alistipes* UO	*Parabacteroides* AT	*Taibaiella* UB	Unspecified Chitinophagales (o)	*Capnocytophaga* UB	Unspecified *Flavobacterium*	*Myroides* AT	*Pedobacter* AT	*Sphingobacterium* AT	*Fusobacterium* UB	Unspecified *Fusobacterium*	*Leptotrichia* UB	*Akkermansia* UB	Total OTU
D1S1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2981
D1S2	16	85	0	21	0	90	0	0	0	0	0	0	0	0	41	8	0	0	0	0	0	0	0	0	0	0	179	5266
D1S3	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8594
D1S4	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3922
D1S5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6584
D1S6	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	4	0	0	0	0	0	0	4261
D1S7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12	54	0	0	0	0	0	0	6138
D2S1	0	0	0	0	0	0	176	14	51	0	8	426	28	36	0	0	0	0	16	0	0	0	0	7	34	24	0	4893
D2S2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5049
D2S3	0	0	0	113	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3598
D2S4	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4566
D2S5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	2759
D3S1	0	0	0	0	0	0	0	0	0	169	0	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5484
D3S2	0	0	0	35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	397	0	0	0	0	0	0	8450
D3S3	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4098
D3S4	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	4094
D3S5	0	0	0	0	18	0	0	0	0	0	0	0	0	0	0	0	11	15	0	220	0	8	25	0	0	0	0	1229

(o): name of order, AT: ambiguous taxa, UB: uncultured bacterium, UO: uncultured organism

Species OTU Counts within the Firmicutes Phylum

Table B16. Raw OTU counts from QIIME2 for each 16S rDNA community sampled from three human donors over multiple sampling times throughout decomposition (D: donor; S: sample time). OTU counts are shown for species within the Bacilli class of the Firmicutes phylum.

Sample ID	Unspecified *Bacillus*	*Gemella* UB	*Gemella* UO	Unspecified *Gemella*	*Kurthia* AT	*Lysinibacillus* AT	*Savagea* AT	*Sporosarcina* UB	Unspecified *Sporosarcina*	Unspecified Planococcaceae (f)	*Macrococcus* AT	*Staphylococcus sciuri*	Unspecified *Staphylococcus*	Unspecified Staphylococcaceae (f)	Unspecified Bacillales (o)	*Abiotrophia* UB	*Carnobacterium* AT	Unspecified *Carnobacterium*	*Granulicatella* UB	*Enterococcus* AT	Unspecified *Enterococcus*	*Vagococcus* AT	Unspecified *Vagococcus*	Unspecified Enterococcaceae (f)	*Lactobacillus crispatus*	*Lactobacillus fermentum*	*Lactobacillus gasseri*	*Lactobacillus plantarum*	*Lactobacillus* UB	Unspecified *Lactobacillus*	Unspecified Lactobacillaceae (f)	Unspecified *Leuconostoc*	*Streptococcus salivarius* subsp. *thermophilus*	Unspecified *Streptococcus*	Unspecified Lactobacillales (o)	Unspecified Bacilli (c)	Total OTU
D1S1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	42	263	334	0	0	264	0	0	0	0	0	0	2981
D1S2	0	0	0	0	0	5	0	0	0	5	0	0	198	0	4	0	0	2	0	35	0	2028	0	0	65	23	149	0	0	44	0	0	0	0	0	0	5266
D1S3	0	0	0	0	0	7489	0	0	11	9	0	0	2	0	0	0	0	3	0	0	0	442	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8594
D1S4	0	0	0	0	0	48	0	2	45	1316	0	0	0	0	275	0	0	8	0	0	414	125	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3922
D1S5	0	0	0	0	0	34	5	6	645	2488	0	0	0	0	2667	0	0	0	0	0	68	62	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6584
D1S6	0	0	0	0	0	168	0	1	830	421	0	0	0	0	364	0	0	3	0	0	7	93	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4261
D1S7	0	0	0	0	0	587	0	0	1245	565	0	0	0	0	213	0	0	0	0	0	2	299	4	0	0	0	0	0	0	0	0	0	0	0	0	0	6138
D2S1	0	216	23	5	0	0	0	0	0	0	0	0	0	0	0	23	0	0	279	0	0	0	0	0	0	0	0	4	0	0	0	0	42	267	0	0	4893
D2S2	7	0	0	0	0	6	0	0	6	48	0	0	0	0	15	0	0	0	0	0	0	248	0	0	0	0	0	0	0	0	0	5	0	5	0	0	5049
D2S3	0	0	0	0	0	0	0	0	5	140	0	0	0	0	322	0	0	0	0	0	148	83	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3598
D2S4	0	0	0	0	0	12	0	0	37	887	0	0	10	0	1699	0	0	0	0	0	229	297	0	10	0	0	0	3	0	0	4	0	0	0	0	5	4566
D2S5	0	0	0	0	2	15	0	2	34	373	0	0	9	0	1202	0	0	0	0	0	183	92	0	9	0	0	0	0	0	0	2	0	0	0	0	0	2759
D3S1	0	0	21	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	373	0	0	0	0	0	1241	702	7	0	40	334	0	0	180	614	0	0	5484
D3S2	5	0	0	0	705	383	0	0	90	233	0	26	16	0	25	0	5	63	0	0	2	361	3	0	0	0	0	0	0	0	0	0	0	0	0	0	8450
D3S3	0	0	0	0	15	53	0	0	9	280	6	0	2	9	808	0	0	15	0	3	456	607	0	0	0	0	0	0	0	0	0	0	0	0	13	0	4098
D3S4	0	0	0	0	0	3	0	0	13	126	0	0	0	3	92	0	0	0	0	0	62	70	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4094
D3S5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1229

(c): name of class, (o): name of order, (f): name of family, AT: ambiguous taxa, UB: uncultured bacterium, UO: uncultured organism

Table B17. Raw OTU counts from QIIME2 for each 16S rDNA community sampled from three human donors over multiple sampling times throughout decomposition (D: donor; S: sample time). OTU counts are shown for species within the Clostridia, Erysipelotrichia, and Negativicutes classes of the Firmicutes phylum.

Sample ID	*Clostridium sensu stricto* 1 AT	*Clostridium sensu stricto* 1 UO	*Clostridium sensu stricto* 7 AT	*Hathewaya* AT	Unspecified *Proteiniclasticum*	Unspecified Clostridiaceae 1 (f)	*Gallicola* UB	Unspecified *Helcococcus*	*Parvimonas* AT	*Peptoniphilus* AT	*Tissierella* AT	*Tissierella* UB	Unspecified *Tissierella*	Family XI W5053 (g) AT	Unspecified Family XI W5053 (g)	Unspecified Family XI (f)	*Eubacterium nodatum* group AT	*Hungatella* UO	Unspecified *Hungatella*	*Stomatobaculum* UB	*Cryptanaerobacter* UB	Unspecified *Paraclostridium*	*Peptostreptococcus* UB	Unspecified Peptostreptococcaceae (f)	Unspecified Clostridiales (o)	Unspecified Clostridia (c)	Unspecified *Erysipelothrix*	Unspecified *Megasphaera*	*Veillonella* UO	Unspecified *Veillonella*	Unspecified Firmicutes (p)	Total OTU
D1S1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2981
D1S2	0	5	4	0	3	0	0	0	0	25	0	0	0	0	0	0	0	14	10	0	0	26	0	0	0	0	0	0	0	0	0	5266
D1S3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0	8594
D1S4	0	0	0	0	0	0	0	437	0	0	0	336	168	0	0	7	0	0	0	0	0	13	129	0	0	0	20	0	0	0	0	3922
D1S5	0	0	0	0	0	0	2	22	0	0	0	95	86	2	0	13	0	0	0	0	0	0	11	0	0	0	9	0	0	0	0	6584
D1S6	0	0	0	0	0	0	0	9	0	0	0	6	10	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	4261
D1S7	0	0	0	0	0	0	0	8	0	0	0	4	5	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	6138
D2S1	0	824	0	195	0	0	0	0	8	0	0	0	0	0	0	0	5	0	0	19	0	0	0	0	0	0	0	7	14	30	0	4893
D2S2	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	113	0	12	0	0	0	0	0	0	0	5049
D2S3	0	17	14	63	0	0	23	559	0	46	31	486	620	26	0	71	0	0	0	0	53	24	41	4	36	10	44	0	0	0	9	3598
D2S4	0	3	11	27	0	0	12	273	0	48	13	55	279	30	0	23	0	0	0	0	4	7	25	0	25	2	24	0	0	0	0	4566
D2S5	0	0	28	37	0	0	7	376	0	58	14	25	112	23	3	8	0	0	0	0	3	1	20	3	5	0	5	0	0	0	2	2759
D3S1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	309	0	5484
D3S2	0	0	0	0	2	0	9	27	0	10	5	0	2	2	0	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	8450
D3S3	2	0	24	0	0	2	55	462	0	364	50	3	107	23	15	5	0	0	0	0	2	0	314	0	0	0	9	0	0	0	0	4098
D3S4	3	0	2	3	0	3	2	8	0	24	0	0	0	4	2	0	0	0	0	0	0	0	15	0	0	0	0	0	0	0	0	4094
D3S5	0	0	0	0	0	0	0	2	0	2	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	1229

(p): name of phylum, (c): name of class, (o): name of order, (f): name of family, (g) name of genus, AT: ambiguous taxa, UB: uncultured bacterium, UO: uncultured organism

Species OTU Counts within the Proteobacteria Phylum

Table B18. Raw OTU counts from QIIME2 for each 16S rDNA community sampled from three human donors over multiple sampling times throughout decomposition (D: donor; S: sample time). OTU counts are shown for species within the Alphaproteobacteria, Betaproteobacteria, Deltaproteobacteria, Epsilonproteobacteria, and Oligoflexia classes of the Proteobacteria phylum.

Sample ID	Uncultured Micavibrionales (o)	*Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* AT	*Pseudochrobactrum* AT	*Pseudochrobactrum* UB	Unspecified *Acidovorax*	*Aquabacterium* AT	Unspecified *Aquabacterium*	*Comamonas* AT	*Paenalcaligenes* AT	*Undibacterium* UB	Unspecified Burkholderiaceae (f)	Unspecified *Neisseria*	*Vitreoscilla* AT	*Azospira* UB	Unspecified Betaproteobacteriales (o)	*Bdellovibrio* AT	*Arcobacter* AT	*Campylobacter* UO	*Oligoflexus* UB	Total OTU
D1S1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2981
D1S2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5266
D1S3	0	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	8594
D1S4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3922
D1S5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6584
D1S6	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	4261
D1S7	0	0	0	5	0	0	0	0	44	0	0	0	0	0	0	0	0	0	0	6138
D2S1	0	0	0	0	0	0	0	0	0	0	0	409	0	0	0	0	0	15	0	4893
D2S2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5049
D2S3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3598
D2S4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4566
D2S5	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	2759
D3S1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	87	0	5484
D3S2	0	0	0	0	0	0	0	24	0	0	0	0	8	0	0	0	0	0	0	8450
D3S3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4098
D3S4	0	0	0	0	0	0	0	30	0	0	0	0	0	0	0	0	0	0	0	4094
D3S5	5	14	9	0	13	56	5	29	0	160	7	0	0	10	143	11	16	0	106	1229

(o): name of order, (f): name of family, AT: ambiguous taxa, UB: uncultured bacterium, UO: uncultured organism

Table B19. Raw OTU counts from QIIME2 for each 16S rDNA community sampled from three human donors over multiple sampling times throughout decomposition (D: donor; S: sample time). OTU counts are shown for species within the Gammaproteobacteria class of the Proteobacteria phylum.

Sample ID	*Ignatzschineria* AT	*Wohlfahrtiimonas chitiniclastica*	Unspecified *Morganella*	*Pantoea* UB	*Proteus* AT	*Providencia* AT	Unspecified *Providencia*	Unspecified Enterobacteriaceae (f)	*Legionella* AT	*Haemophilus haemolyticus*	*Haemophilus* UO	*Acinetobacter* AT	*Acinetobacter* UB	Unspecified *Acinetobacter*	Unspecified *Alkanindiges*	*Pseudomonas* UB	Unspecified *Pseudomonas*	Unspecified Pseudomonadaceae (f)	Unspecified *Stenotrophomonas*	Unspecified Gammaproteobacteria (c)	Total OTU
D1S1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2981
D1S2	1431	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5266
D1S3	429	19	86	0	26	38	5	0	0	0	0	0	0	0	0	0	4	0	0	0	8594
D1S4	550	13	2	0	2	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0	3922
D1S5	268	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	99	0	0	0	6584
D1S6	262	0	0	0	4	2	0	0	0	0	0	0	0	21	0	0	2037	7	0	2	4261
D1S7	1057	3	3	0	3	2	0	0	0	0	0	20	0	106	0	8	1875	0	0	3	6138
D2S1	0	0	0	0	0	0	0	0	0	28	439	0	0	0	0	0	582	0	0	0	4893
D2S2	1901	2574	35	0	4	12	0	25	0	0	0	0	0	0	0	0	0	0	0	0	5049
D2S3	509	89	2	0	2	4	0	0	0	0	0	0	0	2	0	0	2	0	0	0	3598
D2S4	308	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	196	0	0	0	4566
D2S5	35	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	54	0	0	0	2759
D3S1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5484
D3S2	740	10	2	0	4	11	0	0	0	0	0	1386	5	3800	0	0	15	0	0	0	8450
D3S3	160	0	0	0	9	19	0	3	0	0	0	0	0	6	0	0	0	0	0	0	4098
D3S4	1038	0	2	5	6	33	0	15	0	0	0	23	0	21	5	0	15	0	2	0	4094
D3S5	2	0	0	0	0	0	0	89	12	0	0	31	0	24	0	0	129	0	3	0	1229

(c): name of class, (f): name of family, AT: ambiguous taxa, UB: uncultured bacterium, UO: uncultured organism

APPENDIX C: RESULTS FOR THE METABOLIC TESTING OF ISOLATES

Table C1. General microbiology culture environmental parameters, spore and capsule formation, and motility testing.

Original Sample ID	Genus	Species	Temperature Tolerance (°C)	Salt Tolerance (%)	pH Tolerance	Facultative Anaerobe	Terminal Spores	Central Spores	Motility Test	SIM Motility	Motility Hanging Drop Assay	Flagella Present	Flagella Number	Flagella Location	Capsule Present
D3S4	*Acinetobacter*	*baumanii*	10 - 47	0 - 1	5 - 9	+			-		+	-	0		
D1S7	*Acinetobacter*	*guillouiae*	4 - 47	0 - 7.5	4 - 10	+			-		+	-	0		+
D1S1	*Arthrobacter*	*nicotinovorans*	4 - 37	0 - 7.5	4 - 10	+			-						
D1S2	*Arthrobacter*	*nitroguajacolicus*	4 - 47	0 - 7.5	4 - 10	+			-						
D1S2	*Arthrobacter*	*nitroguajacolicus*	4 - 47	0 - 7.5	4 - 10	+			-						
D1S2	*Arthrobacter*	*nitroguajacolicus*	4 - 37	0 - 7.5	4 - 10	-			-						
D1S2	*Bacillus*	*cereus*	15 - 47	0 - 7.5	4 - 10	+		+	-		+	-	0		+
D1S2	*Bacillus*	*cereus*	10 - 47	0 - 7.5	4 - 10	+		+	-		+				+
D1S4	*Bacillus*	*cereus*	10 - 47	0 - 7.5	4 - 10	+			+			-	0		
D1S6	*Bacillus*	*cereus*	10 - 42	0 - 7.5	4 - 10	+	+		-						+
D1S7	*Bacillus*	*cereus*	10 - 47	0 - 7.5	4 - 10	+	+	+	-						+
D2S5	*Bacillus*	*cereus*	15 - 42	0 - 7.5	4 - 10	+		+	-		+	+	3	terminal	
D2S4	*Bacillus*	*mycoides*	10 - 47	0 - 7.5	4 - 10	+		+	-		-				+
D2S5	*Bacillus*	*simplex or muralis*	4 - 42	0 - 15	4 - 10	-			-						
D3S5	*Comamonas*	*terrigena*	4 - 42	0 - 1	5 - 10	+			+			-	0		
D3S1	*Corynebacterium*	*striatum*	25 - 42	0 - 7.5	7 - 10	+			-						
D3S1	*Dermacoccus*	*nishinomiyaensis*	25 - 37	0 - 7.5	4 - 10	+									
D1S5	*Dermacoccus*	*nishinomiyaensis*	15 - 47	0 - 15	3 - 10	-			-						
D3S1	*Enhydrobacter*	*aerosaccus*	10 - 42	1	7	+			-		-	-	0		
D3S1	*Enterococcus*	*faecalis*	10 - 51	0 - 10	4 - 10	+			-		-	-	0		
D2S2	*Hafnia*	*paraluei*	4 - 47	0 - 7.5	5 - 10	+			+		+	+	2.5	terminal	
D3S2	*Kurthia*	*zopfii*	4 - 42	0 - 5	4 - 9	+			-			+	2.5	both poles	
D3S1	*Lactobacillus*	*paracasei*	4 - 42	0 - 1	4 - 9	-			-		+	-	0		
D3S1	*Lactobacillus*	*pentosus*	25 - 37	0 - 5	4 - 10	+			+		-				
D3S4	*Lysinibacillus*	*fusiforms*	10 - 42	0 - 7.5	4 - 10	-	+		-						
D1S5	*Macrococcus*	*caeolyticus*	10 - 47	0 - 7.5	4 - 10	-			-						
D3S1	*Massilia*	sp. WG5	10 - 47	0 - 1	4 - 10	-			-		+	+	1	terminal	

Table C2. General microbiology culture environmental parameters, spore and capsule formation, and motility testing (cont.).

Original Sample ID	Genus	Species	Temperature Tolerance (°C)	Salt Tolerance (%)	pH Tolerance	Facultative Anaerobe	Terminal Spores	Central Spores	Motility Test	SIM Motility	Motility Hanging Drop Assay	Flagella Present	Flagella Number	Flagella Location	Capsule Present
D1S1	*Microbacteriacae*	*bacterium*	15 - 42	0 - 7.5	4 - 10	-			-		+				
D1S2	*Micrococcus*	*aloeverae*	4 - 37	0 - 7.5	4 - 10	+			-						
D1S3	*Morganella*	*morganii*	10 - 42	0 - 7.5	4 - 10	+			+						+
D1S4	*Morganella*	*morganii*	10 - 47	0 - 7.5	3 - 10	+			+	-	-	+	2	terminal	-
D3S2	*Morganella*	*morganii*	10 - 37	0 - 5	4 - 9	+			+		+	+	1	terminal	
D1S6	*Myroides*	*profundi*	10 - 25	0 - 7.5	4 - 10	+			-		-				
D2S1	*Nocardia*	*coeliaca*	4 - 25	0 - 1	4 - 10	-			-						
D2S1	*Paenarthrobacter*	*nicotinovorans*	10 - 42	0 - 7.5	4 - 10	+			-						
D1S3	*Proteus*	*vulgaris*	10 -47	0 - 7.5	4 - 10	+			+		+	-	0		-
D1S3	*Proteus*	*vulgaris*	4 - 50	0 - 10	4 - 10	+			+	+	+	+	1.5	everywhere	
D1S3	*Proteus*	*vulgaris*	10 - 42	0 - 7.5	4 - 10	+			+	+	+	+	1	terminal	
D1S4	*Proteus*	*vulgaris*	10 - 37	0 - 7.5	4 - 10	-			+	+	+	-	0		-
D1S6	*Proteus*	*vulgaris*	10 - 42	0 - 7.5	4 - 10	+			+			-	0		
D2S3	*Providencia*	*alcalifaciens*	15 - 47	0 - 7.5	4 - 10	+			+		+	+	1.5	terminal	
D3S3	*Providencia*	*rustigianii*	10 - 42	0 - 7.5	4 - 10	+			+	-					
D3S3	*Providencia*	*vermicola*	10 - 47	0 - 7.5	4 - 10	+			+	+					
D2S1	*Pseudomonas*	*koreensis*	4 - 47	0 - 5	4 - 10	-			-		+	-			-
D2S1	*Pseudomonas*	*koreensis*	4 - 37	0 - 7.5	4 - 10	+			+						
D2S1	*Pseudomonas*	*moraviensis*	4 - 42	0 - 7.5	4 - 10	+			+		+				
D3S5	*Raoultella*	*terrigena*	4 - 47	0 - 7.5	4 - 10	+			-	-					+
D2S2	*Serratia*	*liquefaciens*	4 - 47	0 - 7.5	5 - 10	+			+		+	+	2	terminal	
D1S1	*Staphylococcus*	*saprophyticus*	10 - 47	0 - 15	4 - 10	+			-						
D2S3	*Staphylococcus*	*sciuri*	4 - 47	0 - 15	5 - 10	+			-						
D3S2	*Staphylococcus*	*surius*	4 - 45	0 - 15	7 - 9	+			-						
D2S4	*Staphylococcus*	*xylosus*	10 - 47	0 - 15	4 - 10	+			-						
D2S5	*Staphylococcus*	*xylosus*	10 - 45	0 - 15	4 - 10	+			-						

Table C3. General microbiology culture fermentation substrates.

Original Sample ID	Culture ID	Genus	Species	Adonitol	Arabinose	Dulcitol	Mannitol	Sorbitol	Sucrose	Glucose Fermentation		Gas Production from Glucose Fermentation		Lactose Fermentation				
										ET	TSIA	ET	TSIA	ET	MCA	EMB	TSIA	LM
D3S4	**12B-2**	*Acinetobacter*	*baumanii*	-	-	-		-		-		-		-	+			
D1S7	**22B-1**	*Acinetobacter*	*guillouiae*	+	+	-		+		-		-		+	+			
D1S1	**15B-3**	*Arthrobacter*	*nicotinovorans*	-	-	-		-		-		-		-				
D1S2	**07A-4**	*Arthrobacter*	*nitroguajacolicus*	-	-	-	+	-	I	+	+	-	-	-			-	
D1S2	**07B-3**	*Arthrobacter*	*nitroguajacolicus*	-	-	-		-		-		-		-				
D1S2	**17B-3**	*Arthrobacter*	*nitroguajacolicus*	-	-	-		-		-		-		-	+			
D1S2	**17A-2**	*Bacillus*	*cereus*	-	-	-		-	I	+	+	-	-	-			-	
D1S2	**17B-1**	*Bacillus*	*cereus*	-	-	-		-	I	-	I	-	-	-			-	
D1S4	**19B-1**	*Bacillus*	*cereus*	-	-	-		-	I	+	+	-	-	-	+		-	
D1S6	**21A-1**	*Bacillus*	*cereus*	-	-	-		-		+		-		-				
D1S7	**22A-2**	*Bacillus*	*cereus*	-	-	-		-		+		-		-				
D2S5	**06B-1**	*Bacillus*	*cereus*	-	-	-		-		+		-		-				
D2S4	**05A-2**	*Bacillus*	*mycoides*	-	-	-		-		+		-		-				
D2S5	**06A-1**	*Bacillus*	*simplex or muralis*	-	-	-	-	-		-		-		-				
D3S5	**13A-3**	*Comamonas*	*terrigena*	-	-	-		-		+		-		-				
D3S1	**09B-2**	*Corynebacterium*	*striatum*	-	-	-		-		+		-		-				
D3S1	**09C-1**	*Dermacoccus*	*nishinomiyaensis*	-	-	-		-		-		-		-				
D1S5	**20C-1**	*Dermacoccus*	*nishinomiyaensis*	-	-			-		-		-		-				
D3S1	**08A-2**	*Enhydrobacter*	*aerosaccus*	-	-	-		-	-	-	-	-	-	-			-	
D3S1	**09A-3**	*Enterococcus*	*faecalis*	-	+			+		+		-		+				
D2S2	**03B-2**	*Hafnia*	*paraluei*	-	-	-		-	I	+	+	+	+	-	+	+	-	
D3S2	**10B-2**	*Kurthia*	*zopfii*	-	-	-		-		-		-		-				
D3S1	**08A-1**	*Lactobacillus*	*paracasei*	+	+	+		+		+		-		+				+
D3S1	**09C-2**	*Lactobacillus*	*pentosus*	+	+			+		+		-		+				-
D3S4	**12A-2**	*Lysinibacillus*	*fusiforms*	-	-	-		-		-		-		-				
D1S5	**20A-3**	*Macrococcus*	*caeolyticus*	-	-	-	-	-		-		-		-				
D3S1	**08B-2**	*Massilia*	sp. WG5	-	-	-	-	-		+		-		-				

ET: enteropluri tube, TSIA: triple sugar iron agar, MCA: MacConkey agar, EMB: eosin-methylene blue media, LM: litmus milk media, I: inconclusive

Table C4. General microbiology culture fermentation substrates (cont.).

Original Sample ID	Culture ID	Genus	Species	Adonitol	Arabinose	Dulcitol	Mannitol	Sorbitol	Sucrose	Glucose Fermentation		Gas Production from Glucose Fermentation		Lactose Fermentation				
										ET	TSIA	ET	TSIA	ET	MCA	EMB	TSIA	LM
D1S1	**16A-2**	*Microbacteriacae*	*bacterium*	+	+	-		-		+		-		-				
D1S2	**17A-1**	*Micrococcus*	*aloeverae*	-	-	-		-		-		-		-				
D1S3	**18B-1**	*Morganella*	*morganii*	-	-			-		+		+		-	+	+		
D1S4	**19A-1**	*Morganella*	*morganii*	+	+			+	I	+	+	-	+	+	+	+	+	
D3S2	**10B-1**	*Morganella*	*morganii*	-	-			-		+		+		-	+	+		
D1S6	**21B-3**	*Myroides*	*profundi*	-	-	-		-		+		-		-				
D2S1	**01B-2**	*Nocardia*	*coeliaca*	-	-	-		-		+		-		-				
D2S1	**02A-1**	*Paenarthrobacter*	*nicotinovorans*	-	-	-		-		-		-		-				
D1S3	**18A-2**	*Proteus*	*vulgaris*	-	-		-	-	I	+	+	-	-	-	+		-	
D1S3	**14A-3**	*Proteus*	*vulgaris*	-	-			-	I	+	+	+	-	-	+		-	
D1S3	**14B-1**	*Proteus*	*vulgaris*	-	-	-		-	I	+	+	+	-	-	+		-	
D1S4	**19B-1**	*Proteus*	*vulgaris*	-	-	-		-	I	+	+	+	-	-	+		-	
D1S6	**21A-2**	*Proteus*	*vulgaris*	-	-			-	I	+	+	-	-	-			-	
D2S3	**04B-2**	*Providencia*	*alcalifaciens*	-	-			-		+		-		-	+	+		
D3S3	**11B-2**	*Providencia*	*rustigianii*	-	-			-		+		-		-	-	-		
D3S3	**11A-3**	*Providencia*	*vermicola*	-	-		-	-	I	+	+	-	-	-	+	+	-	
D2S1	**01A-2**	*Pseudomonas*	*koreensis*	-	-	-		-		+		-		-	+			-
D2S1	**02A-2**	*Pseudomonas*	*koreensis*	-	-	-	-	-		-		-		-	+			-
D2S1	**02B-2**	*Pseudomonas*	*moraviensis*	-	-	-	-	-		-		-		-	+			+
D3S5	**13B-2**	*Raoultella*	*terrigena*	-	-	-	-	-	I	+	+	+	+	-	+	+	-	
D2S2	**03A-1**	*Serratia*	*liquefaciens*	-	-	-		-		+		+		-	+	+		
D1S1	**16A-2**	*Staphylococcus*	*saprophyticus*	-	-	-	+	-		+		+		+				
D2S3	**04A-2**	*Staphylococcus*	*sciuri*	-	+	-	+	+		+		-		-				
D3S2	**10A-1**	*Staphylococcus*	*surius*	-	-	-	+	+		+		-		-				
D2S4	**05B-2**	*Staphylococcus*	*xylosus*	-	+	-	+	-		+		-		-				
D2S5	**06A-3**	*Staphylococcus*	*xylosus*	-	-	-	+	-		+		-		-				

ET: enteropluri tube, TSIA: triple sugar iron agar, MCA: MacConkey agar, EMB: eosin-methylene blue media, LM: litmus milk media, I: inconclusive

Table C5. General microbiology culture enzyme activity.

Original Sample ID	Culture ID	Genus	Species	Hemolysis	Caseinase	Casein Coagulation	Catalase Test	Coagulase	DNase	Esculinase	Gelatinase	Lecithinase	Lipase	Oxidase Test	Peptone Catabolism	Starch-Hydrolyzing	Urease
D3S4	**12B-2**	*Acinetobacter*	*baumanii*				-							-			+
D1S7	**22B-1**	*Acinetobacter*	*guillouiae*	β			+				+			-			+
D1S1	**15B-3**	*Arthrobacter*	*nicotinovorans*				+						-	-		-	+
D1S2	**07A-4**	*Arthrobacter*	*nitroguajacolicus*		+		+				+		+	-	-	-	+
D1S2	**07B-3**	*Arthrobacter*	*nitroguajacolicus*	γ	+		+				-		+	-		-	+
D1S2	**17B-3**	*Arthrobacter*	*nitroguajacolicus*	γ	+		+						+	-		-	+
D1S2	**17A-2**	*Bacillus*	*cereus*	β	+		-				+	+		+	-	-	+
D1S2	**17B-1**	*Bacillus*	*cereus*				-			+	+	+		+	-	-	+
D1S4	**19B-1**	*Bacillus*	*cereus*	γ			+				+			+	-		+
D1S6	**21A-1**	*Bacillus*	*cereus*	β			+							+		-	+
D1S7	**22A-2**	*Bacillus*	*cereus*	β			+					+		+		-	+
D2S5	**06B-1**	*Bacillus*	*cereus*				+					+		+		+	+
D2S4	**05A-2**	*Bacillus*	*mycoides*	β			+				+	+		+		+	+
D2S5	**06A-1**	*Bacillus*	*simplex or muralis*				+		-			-		+		-	+
D3S5	**13A-3**	*Comamonas*	*terrigena*	γ			-				-			+			+
D3S1	**09B-2**	*Corynebacterium*	*striatum*	γ			+			-				-		-	+
D3S1	**09C-1**	*Dermacoccus*	*nishinomiyaensis*				+				-			-			+
D1S5	**20C-1**	*Dermacoccus*	*nishinomiyaensis*				+							-			+
D3S1	**08A-2**	*Enhydrobacter*	*aerosaccus*				-							-	+		+
D3S1	**09A-3**	*Enterococcus*	*faecalis*	α			-							-			-
D2S2	**03B-2**	*Hafnia*	*paraluei*				+							-	-		+
D3S2	**10B-2**	*Kurthia*	*zopfii*				+							+			+
D3S1	**08A-1**	*Lactobacillus*	*paracasei*		-	+	-							+			-
D3S1	**09C-2**	*Lactobacillus*	*pentosus*		-	+	-							+			-
D3S4	**12A-2**	*Lysinibacillus*	*fusiforms*				-				+			+		+	+
D1S5	**20A-3**	*Macrococcus*	*caeolyticus*	γ			+		-	-	+		+	+			+
D3S1	**08B-2**	*Massilia*	sp. WG5		-		+		-	+	+		+	-			+

Table C6. General microbiology culture enzyme activity (cont.).

Original Sample ID	Culture ID	Genus	Species	Hemolysis	Caseinase	Casein Coagulation	Catalase Test	Coagulase	DNase	Esculinase	Gelatinase	Lecithinase	Lipase	Oxidase Test	Peptone Catabolism	Starch-Hydrolyzing	Urease
D1S1	**16A-2**	*Microbacteriacae*	*bacterium*				-				-			-			-
D1S2	**17A-1**	*Micrococcus*	*aloeverae*	γ			+							+			+
D1S3	**18B-1**	*Morganella*	*morganii*	α			-							-			+
D1S4	**19A-1**	*Morganella*	*morganii*	γ			+							-	-		+
D3S2	**10B-1**	*Morganella*	*morganii*				+							-			+
D1S6	**21B-3**	*Myroides*	*profundi*	β			+						+	+			+
D2S1	**01B-2**	*Nocardia*	*coeliaca*	γ	-		+			+			+	-		+	+
D2S1	**02A-1**	*Paenarthrobacter*	*nicotinovorans*				+						+	-		+	+
D1S3	**18A-2**	*Proteus*	*vulgaris*	γ			-							-	-		+
D1S3	**14A-3**	*Proteus*	*vulgaris*	γ			+							-	-		+
D1S3	**14B-1**	*Proteus*	*vulgaris*				+							-	-		+
D1S4	**19B-1**	*Proteus*	*vulgaris*	β	-		-							-	-		+
D1S6	**21A-2**	*Proteus*	*vulgaris*	β	+		+			+	+			-	-	-	+
D2S3	**04B-2**	*Providencia*	*alcalifaciens*				+							-			+
D3S3	**11B-2**	*Providencia*	*rustigianii*				-							-			+
D3S3	**11A-3**	*Providencia*	*vermicola*	γ			-				-			-	-		+
D2S1	**01A-2**	*Pseudomonas*	*koreensis*		-	-	+				+		-	+		+	+
D2S1	**02A-2**	*Pseudomonas*	*koreensis*	γ	-	-	+	-			+		-	+		+	+
D2S1	**02B-2**	*Pseudomonas*	*moraviensis*	γ	+	+	+				+	+	-	+		+	+
D3S5	**13B-2**	*Raoultella*	*terrigena*		-		-							-	-		+
D2S2	**03A-1**	*Serratia*	*liquefaciens*	γ			+		+		+			-			+
D1S1	**16A-2**	*Staphylococcus*	*saprophyticus*				+	-	-					-			+
D2S3	**04A-2**	*Staphylococcus*	*sciuri*	γ			+	-	-					+			+
D3S2	**10A-1**	*Staphylococcus*	*surius*	γ			+	-	-		+			+			+
D2S4	**05B-2**	*Staphylococcus*	*xylosus*	γ			+	-	-					-			+
D2S5	**06A-3**	*Staphylococcus*	*xylosus*		+		+	-	-					+			+

Table C7. General microbiology culture nitrate, sulfur, and litmus reduction.

Original Sample ID	Culture ID	Genus	Species	Nitrate Reduction		H_2S Production			Indole Production		Reduction of Litmus
				Nitrite	Gaseous Nitrogen	ET	TSIA	SIM	ET	SIM	
D3S4	**12B-2**	*Acinetobacter*	*baumanii*	-	-	-			-		
D1S7	**22B-1**	*Acinetobacter*	*guillouiae*	-	-	-			-		
D1S1	**15B-3**	*Arthrobacter*	*nicotinovorans*	-	-	+			-		
D1S2	**07A-4**	*Arthrobacter*	*nitroguajacolicus*	+	-	-	-		-		
D1S2	**07B-3**	*Arthrobacter*	*nitroguajacolicus*	-	-	-			-		
D1S2	**17B-3**	*Arthrobacter*	*nitroguajacolicus*	-	-	+			-		
D1S2	**17A-2**	*Bacillus*	*cereus*	-	-	-	-		-		
D1S2	**17B-1**	*Bacillus*	*cereus*	-	-	-	-		-		
D1S4	**19B-1**	*Bacillus*	*cereus*	+	-	-	+	+	-	+	
D1S6	**21A-1**	*Bacillus*	*cereus*	-	-	-			-		
D1S7	**22A-2**	*Bacillus*	*cereus*	-	-	-			-		
D2S5	**06B-1**	*Bacillus*	*cereus*	-	-	-			-		
D2S4	**05A-2**	*Bacillus*	*mycoides*	+	-	-			-		
D2S5	**06A-1**	*Bacillus*	*simplex or muralis*	-	-	-			-		
D3S5	**13A-3**	*Comamonas*	*terrigena*	+	-	-			-		
D3S1	**09B-2**	*Corynebacterium*	*striatum*	-	-	-			-		
D3S1	**09C-1**	*Dermacoccus*	*nishinomiyaensis*	-	-	-			-		
D1S5	**20C-1**	*Dermacoccus*	*nishinomiyaensis*	-	-	-			+		
D3S1	**08A-2**	*Enhydrobacter*	*aerosaccus*	-	-	-	-		-		
D3S1	**09A-3**	*Enterococcus*	*faecalis*	-	-	-			-		
D2S2	**03B-2**	*Hafnia*	*paraluei*	+	-	-	-		-		
D3S2	**10B-2**	*Kurthia*	*zopfii*	-	-	-			-		
D3S1	**08A-1**	*Lactobacillus*	*paracasei*	-	-	-			-		-
D3S1	**09C-2**	*Lactobacillus*	*pentosus*	-	-	-			+		+
D3S4	**12A-2**	*Lysinibacillus*	*fusiforms*	-	-	-			-		
D1S5	**20A-3**	*Macrococcus*	*caeolyticus*	+	-	+			-		
D3S1	**08B-2**	*Massilia*	sp. WG5	+	-	-			-		

ET: enteropluri tube, TSIA: triple sugar iron agar, SIM: sulfur, indole, motility media

Table C8. General microbiology culture nitrate, sulfur, and litmus reduction (cont.).

Original Sample ID	Culture ID	Genus	Species	Nitrate Reduction		H_2S Production			Indole Production		Reduction of Litmus
				Nitrite	Gaseous Nitrogen	ET	TSIA	SIM	ET	SIM	
D1S1	**16A-2**	*Microbacteriacae*	*bacterium*	-	-	-			-		
D1S2	**17A-1**	*Micrococcus*	*aloeverae*	-	-	+			-		
D1S3	**18B-1**	*Morganella*	*morganii*	+	-	+			-		
D1S4	**19A-1**	*Morganella*	*morganii*	+	-	+	-	-	-	-	
D3S2	**10B-1**	*Morganella*	*morganii*	+	-	-			-		
D1S6	**21B-3**	*Myroides*	*profundi*	-	-	-			-		
D2S1	**01B-2**	*Nocardia*	*coeliaca*	-	-	-			-		
D2S1	**02A-1**	*Paenarthrobacter*	*nicotinovorans*	-	-	-			-		
D1S3	**18A-2**	*Proteus*	*vulgaris*	-	-	+	+		-		
D1S3	**14A-3**	*Proteus*	*vulgaris*	+	-	-	+	+	-	+	
D1S3	**14B-1**	*Proteus*	*vulgaris*	+	-	-	+	+	-	+	
D1S4	**19B-1**	*Proteus*	*vulgaris*	+	-	+	+	+	-	-	
D1S6	**21A-2**	*Proteus*	*vulgaris*	-	-	+	-		-		
D2S3	**04B-2**	*Providencia*	*alcalifaciens*	-	-	-			-		
D3S3	**11B-2**	*Providencia*	*rustigianii*	+	-	+		-	-	-	
D3S3	**11A-3**	*Providencia*	*vermicola*	+	-	+	-	-	-	+	
D2S1	**01A-2**	*Pseudomonas*	*koreensis*	-	-	-			-		-
D2S1	**02A-2**	*Pseudomonas*	*koreensis*	-	-	-			-		-
D2S1	**02B-2**	*Pseudomonas*	*moraviensis*	-	-	-			-		-
D3S5	**13B-2**	*Raoultella*	*terrigena*	+	-	-	-	-	-	-	
D2S2	**03A-1**	*Serratia*	*liquefaciens*	+	-	-			-		
D1S1	**16A-2**	*Staphylococcus*	*saprophyticus*	-	-	-			-		
D2S3	**04A-2**	*Staphylococcus*	*sciuri*	+	-	-			-		
D3S2	**10A-1**	*Staphylococcus*	*surius*	+	-	-			-		
D2S4	**05B-2**	*Staphylococcus*	*xylosus*	-	-	-			-		
D2S5	**06A-3**	*Staphylococcus*	*xylosus*	-	-	-			-		

ET: enteropluri tube, TSIA: triple sugar iron agar, SIM: sulfur, indole, motility media

Table C9. General microbiology culture deamination, decarboxylation, alternative carbon utilization, and other tests.

Original Sample ID	Culture ID	Genus	Species	Acetoin Production	Phenylalanine Deamination	Decarboxylation		Citrate Utilization		Utilization of Acetate, Aspartate, Glutamate, and Lactate	Growth on TSA	Growth in Lactic Acid Broth
						Lysine	Ornithine	ET	SC			
D3S4	**12B-2**	*Acinetobacter*	*baumanii*	-	-	-	-	+		-		
D1S7	**22B-1**	*Acinetobacter*	*guillouiae*	+	-	-	-	+		+		
D1S1	**15B-3**	*Arthrobacter*	*nicotinovorans*	-	-	-	-	+				
D1S2	**07A-4**	*Arthrobacter*	*nitroguajacolicus*	-	-	-	-	+	-			
D1S2	**07B-3**	*Arthrobacter*	*nitroguajacolicus*	-	-	-	-	+				
D1S2	**17B-3**	*Arthrobacter*	*nitroguajacolicus*	-	-	-	-	+				
D1S2	**17A-2**	*Bacillus*	*cereus*	-	-	-	-	+				
D1S2	**17B-1**	*Bacillus*	*cereus*	-	-	-	-	+				
D1S4	**19B-1**	*Bacillus*	*cereus*	-	-	-	-	+				
D1S6	**21A-1**	*Bacillus*	*cereus*	-	-	-	-	+				
D1S7	**22A-2**	*Bacillus*	*cereus*	-	-	-	-	+				
D2S5	**06B-1**	*Bacillus*	*cereus*	+	-	-	-	+				
D2S4	**05A-2**	*Bacillus*	*mycoides*	-	-	-	-	+				
D2S5	**06A-1**	*Bacillus*	*simplex or muralis*	-	-	-	-	+				
D3S5	**13A-3**	*Comamonas*	*terrigena*	+	-	-	-	+			+	
D3S1	**09B-2**	*Corynebacterium*	*striatum*	+	-	-	-	+	-		+	
D3S1	**09C-1**	*Dermacoccus*	*nishinomiyaensis*	+	-	-	-	+				
D1S5	**20C-1**	*Dermacoccus*	*nishinomiyaensis*	-	+	-	-	+				
D3S1	**08A-2**	*Enhydrobacter*	*aerosaccus*	-	-	-	-	-				
D3S1	**09A-3**	*Enterococcus*	*faecalis*	+	+	-	-	-				
D2S2	**03B-2**	*Hafnia*	*paraluei*	+	-	+	+	+				
D3S2	**10B-2**	*Kurthia*	*zopfii*	-	-	-	-	+				
D3S1	**08A-1**	*Lactobacillus*	*paracasei*	+	-	-	-	-				-
D3S1	**09C-2**	*Lactobacillus*	*pentosus*	-	+	-	-	-				+
D3S4	**12A-2**	*Lysinibacillus*	*fusiforms*	+	-	-	-	+				
D1S5	**20A-3**	*Macrococcus*	*caeolyticus*	+	-	-	-	+				
D3S1	**08B-2**	*Massilia*	sp. WG5	-	-	-	-	+			+	

ET: enteropluri tube, SC: Simmons' citrate media, TSA: tryptic soy agar

Table C10. General microbiology culture deamination, decarboxylation, alternative carbon utilization, and other tests (cont.).

Original Sample ID	Culture ID	Genus	Species	Acetoin Production	Phenylalanine Deamination	Decarboxylation		Citrate Utilization		Utilization of Acetate, Aspartate, Glutamate, and Lactate	Growth on TSA	Growth in Lactic Acid Broth
						Lysine	Ornithine	ET	SC			
D1S1	**16A-2**	*Microbacteriacae*	*bacterium*	+	-	-	-	-				
D1S2	**17A-1**	*Micrococcus*	*aloeverae*	-	-	-	-	+				
D1S3	**18B-1**	*Morganella*	*morganii*	-	+	-	+	+			+	
D1S4	**19A-1**	*Morganella*	*morganii*	-	+	-	+	+			+	
D3S2	**10B-1**	*Morganella*	*morganii*	-	+	-	+	+			+	
D1S6	**21B-3**	*Myroides*	*profundi*	-	-	-	+	+			+	
D2S1	**01B-2**	*Nocardia*	*coeliaca*	-	-	-	-	+				
D2S1	**02A-1**	*Paenarthrobacter*	*nicotinovorans*	-	-	-	-	+	-			
D1S3	**18A-2**	*Proteus*	*vulgaris*	+	+	-	-	+				
D1S3	**14A-3**	*Proteus*	*vulgaris*	-	+	-	-	+	-			
D1S3	**14B-1**	*Proteus*	*vulgaris*	+	-	-	-	+				
D1S4	**19B-1**	*Proteus*	*vulgaris*	+	-	-	-	+				
D1S6	**21A-2**	*Proteus*	*vulgaris*	+	+	-	-	+				
D2S3	**04B-2**	*Providencia*	*alcalifaciens*	+	+	-	-	+				
D3S3	**11B-2**	*Providencia*	*rustigianii*	-	+	-	-	+				
D3S3	**11A-3**	*Providencia*	*vermicola*	-	+	-	-	+				
D2S1	**01A-2**	*Pseudomonas*	*koreensis*	-	-	-	-	+				
D2S1	**02A-2**	*Pseudomonas*	*koreensis*	-	-	-	-	+				
D2S1	**02B-2**	*Pseudomonas*	*moraviensis*	-	-	-	-	+				
D3S5	**13B-2**	*Raoultella*	*terrigena*	+	-	+	-	+				
D2S2	**03A-1**	*Serratia*	*liquefaciens*	+	-	+	+	+				
D1S1	**16A-2**	*Staphylococcus*	*saprophyticus*	+	-	-	-	+				
D2S3	**04A-2**	*Staphylococcus*	*sciuri*	-	-	-	-	+				
D3S2	**10A-1**	*Staphylococcus*	*surius*	+	-	-	-	+				
D2S4	**05B-2**	*Staphylococcus*	*xylosus*	-	-	-	-	+				
D2S5	**06A-3**	*Staphylococcus*	*xylosus*	-	-	-	-	+				

ET: enteropluri tube, SC: Simmons' citrate media, TSA: tryptic soy agar

Table C11. General microbiology antibiotic resistance of cultures.

Original Sample ID	Culture ID	Genus	Species	Amoxicillin	Ampicillin	Chloramphenicol	Ciprofloxacin	Clindamycin	Colistin	Erythromycin	Nalidixic Acid	Neosporin	Penicillin	Streptomycin	Tetracycline
D3S4	**12B-2**	*Acinetobacter*	*baumanii*			+									
D1S7	**22B-1**	*Acinetobacter*	*guillouiae*						+						
D1S2	**17B-3**	*Arthrobacter*	*nitroguajacolicus*	-									-	-	
D1S2	**17A-2**	*Bacillus*	*cereus*										+		
D1S4	**19B-1**	*Bacillus*	*cereus*					+	+			+	+		
D1S6	**21A-1**	*Bacillus*	*cereus*			-							+		
D2S5	**06B-1**	*Bacillus*	*cereus*		+								+		
D2S4	**05A-2**	*Bacillus*	*mycoides*										+		
D2S5	**06A-1**	*Bacillus*	*simplex or muralis*							-					
D3S1	**09B-2**	*Corynebacterium*	*striatum*							-			-		
D2S2	**03B-2**	*Hafnia*	*paraluei*		+						-			-	-
D3S4	**12A-2**	*Lysinibacillus*	*fusiforms*												-
D1S3	**18B-1**	*Morganella*	*morganii*	-											
D1S4	**19A-1**	*Morganella*	*morganii*										-	+	
D2S1	**01B-2**	*Nocardia*	*coeliaca*									+	-		
D2S1	**02A-1**	*Paenarthrobacter*	*nicotinovorans*	-									-		
D1S3	**18A-2**	*Proteus*	*vulgaris*		+										
D1S3	**14A-3**	*Proteus*	*vulgaris*		+						+		+		+
D1S6	**21A-2**	*Proteus*	*vulgaris*					-	+			+	+		
D2S3	**04B-2**	*Providencia*	*alcalifaciens*		+									-	
D2S1	**02A-2**	*Pseudomonas*	*koreensis*	+			-						+		
D2S1	**02B-2**	*Pseudomonas*	*moraviensis*				-								
D3S5	**13B-2**	*Raoultella*	*terrigena*		-										
D2S2	**03A-1**	*Serratia*	*liquefaciens*				-						+		
D2S3	**04A-2**	*Staphylococcus*	*sciuri*							-			-		
D3S2	**10A-1**	*Staphylococcus*	*surius*										-		
D2S4	**05B-2**	*Staphylococcus*	*xylosus*									-			

Table C12. General microbiology culture EcoPlate results.

Original Sample ID	Culture ID	Genus	Species	Alternative Carbon Sources
D1S2	**17B-1**	*Bacillus*	*cereus*	D-Mannitol, 4-Hydroxy Benzoic Acid, Pyruvic Acid, D-Galacturonic Acid, L-Asparagine, L-Serine, D-Glucosamine, D-Glucosaminic Acid, Putrescine
D1S7	**22A-2**	*Bacillus*	*cereus*	none
D2S4	**05A-2**	*Bacillus*	*mycoides*	none
D3S1	**09A-3**	*Enterococcus*	*faecalis*	D-Cellobiose, N-Acetyl-D-Glucosamine, a-Keto Butyric Acid
D1S5	**20A-3**	*Macrococcus*	*caeolyticus*	none
D3S3	**11B-2**	*Providencia*	*rustigianii*	Pyruvic Acid Methyl Ester, L-Asparagine, L-Serine, N-Acetyl-D-Glucosamine, Glycyl-L-Glutamic Acid, Glucose-1-Phosphate, D,L-a-Glycerol Phosphate
D3S3	**11A-3**	*Providencia*	*vermicola*	Pyruvic Acid Methyl Ester, L-Asparagine, L-Serine, N-Acetyl-D-Glucosamine, Glucose-1-Phosphate, D,L-a-Glycerol Phosphate, D-Malic Acid