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Genomic Analysis of the JCCC Campus Compost System

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Abstract
Composting is the aerobic method of using microbes to convert organic waste into a usable soil amendment. Many compost analyses have focused on nutrient testing rather than genomic analysis. However, bacterial microbes play a vital role in the degradation of organic plant matter in the formation of compost soil amendments [1]. Of the studies that have examined microbial life within compost systems, common findings have been that Firmicutes, Actinobacteria, Proteobacteria, and Bacteroidetes are the most prevalent phyla [2, 3, 4]. Further examination of the presence and impact of microbes in the composting process is still needed. Evaluating the microbial life through a multi-step food waste composting system can contribute to the determination of a baseline for similar systems and assist in the understanding of how microbial life contributes to soil amendments.

Cover Page Footnote
The Faculty Mentor for this project was Heather Seitz, Biology.

Johnson County Community College

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1. Introduction

Composting is the aerobic method of using microbes to convert organic waste into a usable soil amendment. Many compost analyses have focused on nutrient testing rather than genomic analysis. However, bacterial microbes play a vital role in the degradation of organic plant matter in the formation of compost soil amendments [1]. Of the studies that have examined microbial life within compost systems, common findings have been that Firmicutes, Actinobacteria, Proteobacteria, and Bacteroidetes are the most prevalent phyla [2, 3, 4]. Further examination of the presence and impact of microbes in the composting process is still needed. Evaluating the microbial life through a multi-step food waste composting system can contribute to the determination of a baseline for similar systems and assist in the understanding of how microbial life contributes to soil amendments.

2. Importance of Compost

Every year, millions of tons of solid waste enter American landfills and contribute to methane emissions and unnecessary land usage [5]. The largest single contributor to landfills is food waste, which is organic matter that is able to be composted [5]. This food waste, coupled with the other products in landfills, leads to landfills being the third largest contributor to methane gas formation in the United States [6]. An increase in composted materials diverted from landfills has the potential to significantly reduce methane emissions associated with organic matter that is disposed of in landfills [7,8].

In addition to contributing to a decrease in methane emissions, composting is an important aspect of soil health and food system stability worldwide [7,9]. Global soil nutrient depletion continues to be a growing problem both in America and abroad [10]. Microorganisms, many of which are extremely available in compost, contribute to the decomposition of dead plant matter and work to recycle soil nutrients [9]. In addition to helping remedy nutrient depletion, compost can also help remove soil-borne plant pathogens from our food systems and assist in food stability and availability [7].

3. Collection and Methods

The collection site is located on the Johnson County Community College (JCCC) campus in Overland Park, Kansas. JCCC uses a multi-step process to prepare compost to be used as a soil additive on the campus farm. Food waste collected by dining services is initially churned by an auger. The food waste is then transported to an in-vessel composting system for approximately two weeks. The product of the vessel system is then cured compost bays for four to six weeks before being moved to the campus farm to rest.

The situation at JCCC is different from what is known due to there being limited data on the changing microbe colonies throughout composting processes. In order to establish a baseline for microbial growth throughout the composting process, we took samples from multiple time points in the JCCC campus composting system. Samples were collected from the following stages in our composting process: pre-compost (mixed food waste from dining services), in-vessel, layered and unlayered bays, and mid-young and oldest farm-ready piles. Each stage, excluding layered-bay, follows the previous stage with no additional inputs. The layered-bay is

created by layering vessel compost with uncomposted food waste from JCCC dining services. Three samples were collected from each stage to ensure an accurate representation of the microbial life in each. Pre-compost samples were collected from a bucket of churned food waste from JCCC's dining services. In-vessel samples were collected from the window of the composting vessel. Samples from the layered and unlayered bays were taken from a depth of approximately 20 centimeters below the surface. Mid-young and Oldest farm-ready samples were taken from a depth of approximately 20 centimeters below the surface.

Genomic isolation was completed according to instructions using a PowerSoil Kit before being sent to a lab for sequencing. To complete genomic analysis, 16S rRNA PCR primers with barcode on the forward primer were used in a PCR using the HotStarTaq Plus Master Mix Kit. Agarose gel electrophoresis was used to determine amplification of PCR products. Samples were then pooled together in equal proportions and purified using calibrated Ampure XP beads before a DNA library was prepared. Genomic DNA sequencing was then completed on an Illumina MiSeq whereby operational taxonomic units (OTUs) were generated. OTUs were then classified using BLASTn in conjunction with RDPII and NCBI databases.

4. Data and Comparison

4.1 Total Bacteria

Total bacteria counts increased marginally between the pre-vessel and vessel stages. Counts then sharply decreased in the non-layered bay before rising slightly in both mid-young and oldest farm-ready piles. The non-layered bay had drastically higher bacteria counts than the non-layered bay, likely due to the addition and layering of uncomposted food waste. Total bacteria counts for each stage are as follows: 331411 in pre-vessel, 342009 in vessel, 235307 in layered bay, 102384 non-layered bay, 125937 mid-young farm-ready, and 152640 in oldest farm-ready. Bacteria counts peaked during the vessel stage and reached their lowest point within the non-layered bay.

The dominant phylum based on the mean of all stages was Firmicutes (48.475%), followed by Actinobacteria (11.307%), Proteobacteria (30.008%), and Bacteroidetes (3.762%). Cyanobacteria (1.755%), Ascomycota (1.336%), and Gemmatimonadetes (1.208%) also have significant populations in some stages of the composting process. Chloroflexi (0.832%), which is commonly mentioned in other compost analyses, has a mean of under one percent in our analysis.

4.2 Firmicutes

Firmicutes represented the largest single phylum to contribute to the microbial life throughout the JCCC composting system. The majority of Firmicutes have Gram-positive cell walls and produce endospores that assist in their survival during harsh conditions [11]. This is likely the explaination to the domination of Firmicutes in many compost microbial analyses [2]. In our system, Firmicutes were the predominant phylum in the vessel (71.56%), layered (55.00%), non-layered (86.18%), and mid-young stages (59.69%).

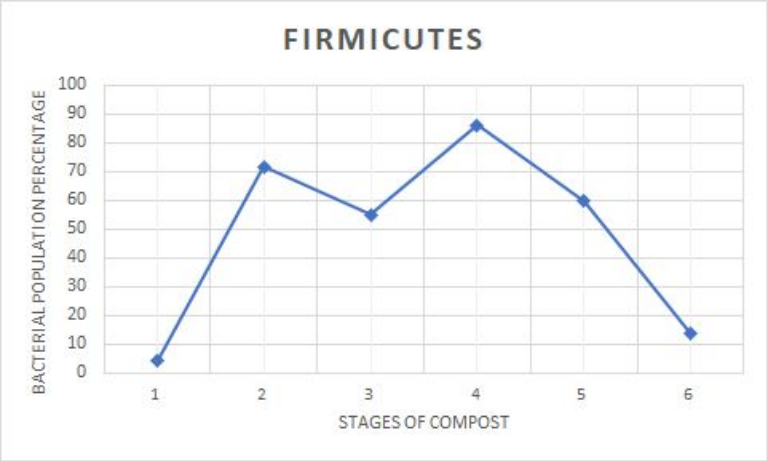

Fig. 1. Firmicutes Percentages Through Stages of Compost
Key: 1=Pre-Vessel, 2=Vessel, 3=Layered Bay, 4=Non-Layered Bay, 5= Mid-Young, 6=Oldest

4.3 Actinobacteria

Actinobacteria, the second most prevalent bacterial phylum represented in the JCCC composting system, is an extremely common phylum that plays a vital role the decomposition of organic matter [12]. Actinobacteria was present in every stage of the JCCC composting system and was the largest phylum in the oldest farm-ready stage (34.01%). Actinobacteria was also found at percentages above one percent in every stage except pre-vessel.

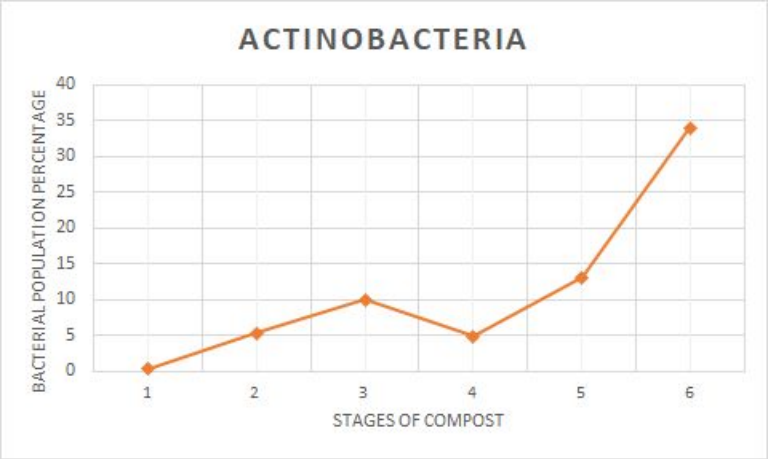

Fig. 2. Actinobacteria Percentages Through Stages of Compost
Key: 1=Pre-Vessel, 2=Vessel, 3=Layered Bay, 4=Non-Layered Bay, 5= Mid-Young, 6=Oldest

4.4 Proteobacteria

Proteobacteria is a phylum of Gram-negative bacteria that are often pathogenic or nitrogen-fixing [13]. Proteobacteria were most prevalent in the pre-vessel, but maintained a strong presence in every stage. Percentages throughout the JCCC composting process are: 81.95% in pre-vessel, 23.00% in vessel, 38.44% in layered bay, 5.76% in non-layered bay, 11.05% in mid-young farm ready, and 23.41% in oldest farm-ready.

One of the most prevailing species within the Proteobacteria phylum was *Salmonella enterica*, a common human pathogen [14]. *S. Entrica* represented 2.01% of total bacterial presence in the pre-vessel stage but was reduced and maintained at 0% throughout the remainder of the process.

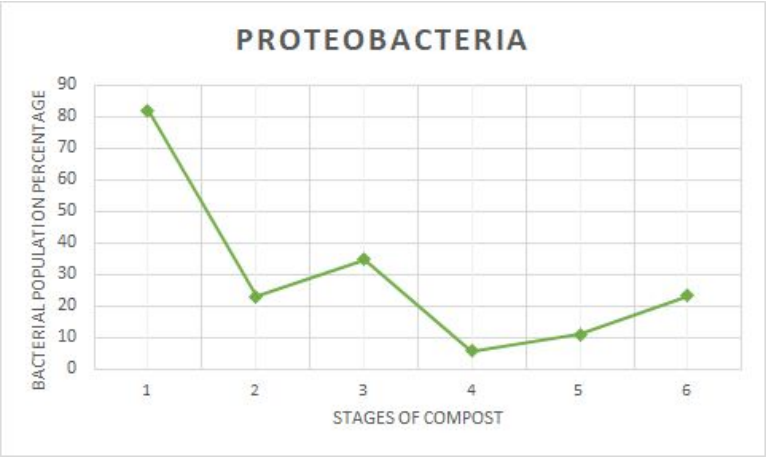

Fig. 3. Proteobacteria Percentages Through Stages of Compost
Key: 1=Pre-Vessel, 2=Vessel, 3=Layered Bay, 4=Non-Layered Bay, 5= Mid-Young, 6=Oldest

4.5 Bacteroidetes

Bacteroidetes are a phylum that colonize in almost every ecosystem [1]. Bacteroidetes are skilled at degrading proteins and carbohydrates [1]. Bacteroidetes are the fourth most common phylum in the JCCC compost system. Of the six stages, Bacteroidetes were most common in the oldest farm-ready stage (10.16%), followed by the mid-young farm-ready (9.15%), non-layered bay (1.90%), and pre-vessel stages (1.24%).

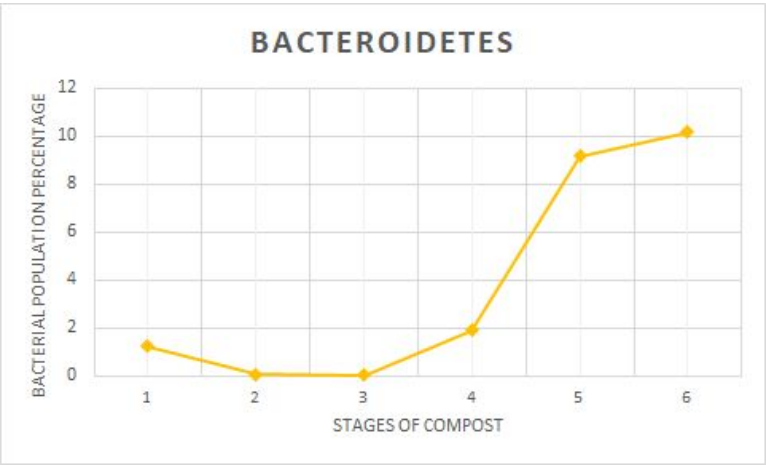

Fig. 4. Bacteroidetes Percentages Through Stages of Compost
Key: 1=Pre-Vessel, 2=Vessel, 3=Layered Bay, 4=Non-Layered Bay, 5= Mid-Young, 6=Oldest

4.6 Comparison

When compared to other bacterial genomic analyses of composts, our system has many similarities and differences. While the JCCC campus composting system has data points from six stages within the system, the analyses from the studies used for comparison contain data from only one stage.

Comparison to chicken manure compost (sample 7 on figure 5) showed the closest relation among our data being within the vessel stage [2]. The chicken manure is composed of 68.00% Firmicutes, 8.7% Actinobacteria, 17.4% Proteobacteria, and 3.9% Bacteroidetes [2]. The vessel stage of our system is composed of 71.56% Firmicutes, 5.35% Actinobacteria, 23.00% Proteobacteria, and 0.08% Bacteroidetes.

Comparison to an agro-industrial waste compost system (sample 8 on figure 5) showed no close relations to one stage of our compost system [3]. However, percentages of Proteobacteria between the agro-industrial waste and the layered bay stage of our system are most similar, with the agro-industrial waste having 39.89% Proteobacteria and the layered bay stage having 34.88% [3]. Actinobacteria populatios are also close between the agro-industrial waste and the oldest farm-ready stage [3].

When compared to a manure compost analysis (sample 9 on figure 5), the oldest farm-ready stage from the JCCC campus most closely related [4]. Both the manure compost and the oldest farm-ready stage are dominated by Actinobacteria, with Proteobacteria and Firmicutes coming second and third, respectively [4].

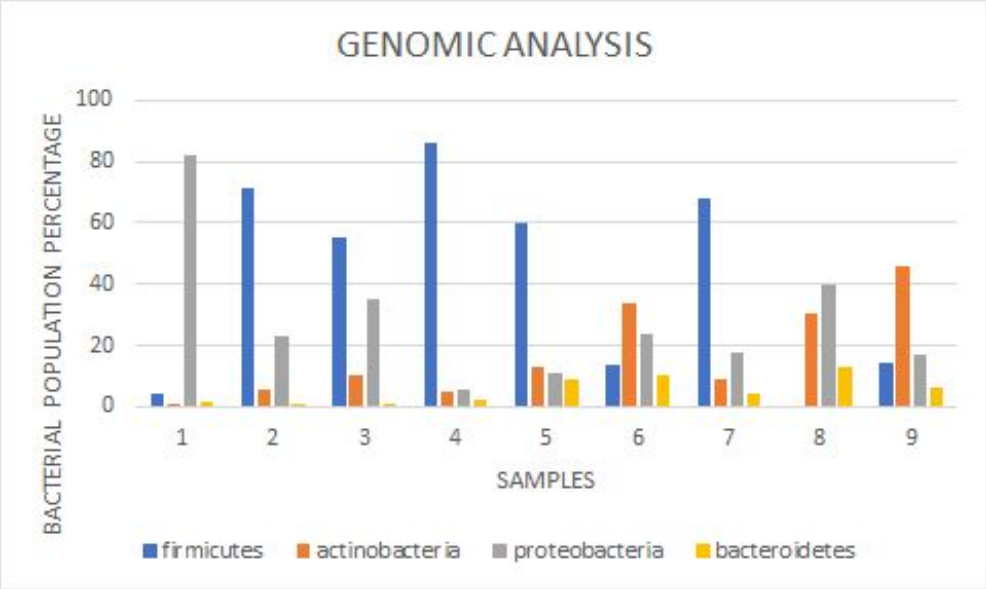

Fig. 5. Phylum Percentages Through Stages of Compost + Three Comparisons
Key: 1=Pre-Vessel, 2=Vessel, 3=Layered Bay, 4=Non-Layered Bay, 5= Mid-Young, 6=Oldest, 7=Chicken Manure Compost (for comparison), 8=Agro-Industrial Waste Compost (for comparison), 9=Manure Compost (for comparison)

Conclusion

The results of our study have concluded that, while Firmicutes, Actinobacteria, Proteobacteria, and Bacteroidetes dominated our samples, the stages of our compost system have distinctly different bacterial profiles. We saw overall increases in each of these phyla, with the exception of Proteobacteria. Furthermore, pathogens such as *Salmonella enterica* were eradicated between the pre-vessel and oldest farm-ready stages of the compost.

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