8-23-2024

Cultivation and genomic analysis of litter decomposition isolates from eastern US temperate forests

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Abstract

Leaf litter decomposition is a relevant and complex process in forest ecosystems that plays a key role in carbon and nutrient cycling and maintaining soil fertility. Bacteria and fungi can decompose the compounds present in leaf litter mostly cellulose, lignin, hemicellulose, pectin and proteins. Microbes are the main producers of enzymes known as Carbohydrate Active Enzymes (CAZymes) allowing them to break down these complex carbohydrates in plant cell walls. Several studies have focused on the different factors affecting decomposition such litter quality of climate. However few studies have evaluated the bacteria and CAZymes activity in leaf litter during decomposition. This study assessed the microbial communities in leaves, and the link between them and the enzyme activities that modulate the decomposition process. We isolated 22 bacteria from leaves from three sites across the eastern U.S. temperate forest. After the 16S rRNA genes analysis, and sanger sequencing we selected three microbes of interest to sequence its whole genome. After the genomic characterization we identified the bacteria *Frondihabitans* and the presence of the CAZymes involved in the breakdown of complex carbohydrates, suggesting that *Frondihabitans* may play a role in carbohydrate metabolism, which may be important for litter decomposition.

Cultivation and genomic analysis of litter decomposition isolates from eastern US temperate forests

by

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Universidad Nacional Autónoma de México, 2016

Thesis
Submitted in partial fulfillment of the requirements for the degree of
Master of Science in Biology.

Syracuse University
August 2024

Acknowledgements

I am extremely grateful to The College of Arts & Sciences at Syracuse University for supporting my studies through a graduate teaching assistantship and Marilyn Sue Kerr Graduate Fellowship.

I am very grateful to my research advisor, Dr. Angela M. Oliverio, for sharing her lab, resources, expertise, and constructive feedback, as well as all the team of Oliverio Lab.

I also wish to thank all the members of my graduate committee: Dr. Heather D. Coleman and Dr. Louis James Lamit for their valuable insight and contributions during and after committee meetings. All their comments were extremely valuable in the completion of this thesis.

Additionally, I would like to thank Dr. Ramesh Raina for serving as the oral chair for my thesis defense, and for his encouragement. I was very fortunate to work alongside him and Dr. Surabhi Raina as a teacher assistant. Thanks to both for all the classes, lessons shared and support.

I want to express my gratitude to Ginny M. Grieb, a lab tech extraordinaire who taught me some lab techniques and always facilitated the material needed.

Finally, and the most important, my family. Thank you so much to my beloved and unforgettable mother Elia, you fought until the end, and gave us unconditional love, happiness, and care. Thanks a lot, to my father Humberto, for giving me love, encouragement and strength. My siblings Alma, Victor, and Humberto, my siblings in law, my nephews, and niece. Lastly, thank you to my professors, and the many friends from Mexico and Syracuse. Without all your support, this would not have been possible.

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Introduction

Litter decomposition is a vital process, where organic matter breaks down releasing carbon dioxide (CO_2) into the atmosphere contributing to the carbon cycle (Gołębiewski, 2019). Decomposition recycles carbon and nutrients in forests since trees and soil can store large amounts of carbon for a long time (Giweta, 2020). The rate of decomposition of the litter determines if forests work like carbon sources (carbon released) or sinks (carbon sequestered), for example, with low decomposition rates carbon in soil is accumulated, while high decomposition rates release more CO_2 (Krishna and Mohan, 2017). The depolymerization and mineralization of organic compounds help to return and incorporate nutrients such as nitrogen, phosphorus, and other elements into the soil, maintaining the nutrient flow in the forests (Xu and Hirarta, 2005). This is an essential process for the nourishment of plants, as it allows them to access the nutrients they need to grow (Haynes, 2012).

The decomposition of litter in forest ecosystems is a complex process that unfolds in four primary stages, as outlined by Adair et al. (2008). The initial phase is characterized by leaching (Krishna and Mohan, 2017). During this leaching phase, a variety of organic substances are extracted from the litter, including sugars, amino acids, and other organic compounds (Adair et al. 2008). The second stage in the decomposition process is more gradual and involves fragmentation by soil animals, chemical alteration by soil microbes, and leaching (Aerts 1997; Chapin, et al. 2011). During this phase, easily degradable (labile) components of the litter are broken down first, leaving behind the more resistant (recalcitrant) materials which persist longer in the ecosystem (Robertson and Paul 2000). The third phase of decomposition involves the

same processes as the second phase but occurs more slowly because the remaining compounds are recalcitrant (difficult to breakdown) such as lignin (Chapin, et al. 2011). Decomposition during the second and third phases is often measured as mass loss from dead leaves. Finally, the phase 4 during decomposition occurs quite slowly and involves the chemical alteration of organic matter that is mixed with mineral soil and the leaching of breakdown products to other soil layers (Schlesinger 1977).

It has been well-documented that the litter decomposition process is also affected by abiotic factors such as soil physicochemical properties, and climate (Suseela, 2019). Physicochemical properties include factors such as pH value, soil material composition, texture, cation exchange capacity (CEC), porosity, and soil organic carbon (SOC) (Giweta, 2020; González, 2002). Climate factors like temperature, precipitation, and evaporation are important regulators of litter decomposition rates by influencing microbial activity (Zhang et al. 2008).

Litter quality of different tree species influences decomposition, hosts, and shapes microbial communities because of variations in the composition of structural molecules, tannins in leaf, and litter chemistry (Santschi et al. 2018). Cellulose, hemicellulose, lignin and tannins are some of their components, and its molecular structure impact significantly litter decomposition rates (Giweta, 2020). Lignin being a complex phenolic polymer makes its degradation more difficult (Katahira et al. 2018). Unlike cellulose an homopolymer made up of glucose units linked by 1,4-glycosidic bonds (Deshavath et al. 2019). The hemicellulose structure consists of heteropolymers like xylose, arabinose, glucose, mannose and galactose (Zhao et al. 2022). The effect

of leaf litter species helps to explain the activity of different decomposers (Sánchez-Galindo, 2021).

Red maple (*Acer rubrum*) for example is a dominant deciduous tree found in different forest of the eastern U.S (Alexander et al. 2014). Red maple success in different sites maybe is related to its response to elevated carbon dioxide by enhancing its photosynthetic activity and increasing biomass production (Abrams, 1998). Moreover, *Acer rubrum* possesses characteristics that affect decomposition rates (Alexander et al. 2014). Its leaf litter is rich in cellulose, which starts at lower levels in early leaf development but increases as the leaves mature throughout the growing season (Kern et al. 2022). Lignin, another key structural component provides rigidity and strength to the leaves, is present in lower amounts than cellulose but rises in concentration in the cell walls as the leaves age, reaching its highest levels before senescence (Alexander, 2014).

The fact that leaf litter has a high-quality means that it is easier for microorganisms to decompose, which implies that the leaf litter has more carbon compounds that are easily degraded. Fierer et al (2003) mentioned that gram-negative bacteria utilize high-quality litter. In contrast to low-quality litter where the amount of nutrients is poor, and there is an accumulation of recalcitrant molecules harder to degrade (Frossard et al. 2013), and according to Bray et al. (2012) the gram-positive fungi and bacteria are the principal decomposers.

Leaf litter chemistry influences the microbial community due to the affinity to carbon substrates and the abundance of certain taxa involved in the decomposition (Frossard et al. 2013). Song et al. (2023) demonstrated that lignin was linked with fungi

positively since they have the ability to decompound complex organic molecules faster than bacteria. The importance of litter chemistry increases while the decomposition progresses (Bradford et al. 2016). However, further research is required to evaluate the litter quality and the composition of microbial communities (García-Palacios et al. 2013).

Biotic factors such as the microbial communities that break down leaf litter influence rates of decomposition. Microorganisms, and particularly the interplay between bacteria and fungi, have a relevant role during the decomposition process and nutrient acquisition (Zhang et al. 2024). The microbial communities of fungi are considered the main litter decomposition drivers (Kjoller and Struwe, 1992), because of several reasons: their rapid succession, the different taxa, and a significant portion of the fungi community present on leaves at the early, intermediate, and late stages during decomposition (Frankland, 1998). For instance, Ascomycetes tend to dominate the early stages of decomposition, being that they can decompose cellulose, while Basidiomycetes increase in relative abundance during later stages since they have a higher ability to degrade lignin (Voříšková and Baldrian, 2013; Gołębiewski, 2019; Schneider et al. 2012). Additionally, fungi can transform various components of litter due to their enzymatic repertoire (Floudas et al. 2020).

Bacterial communities also have a great impact on decomposition, the transformation of chemical compounds, and nutrient release (Frey-Klett et al. 2011; López-Mondéjar et al. 2015) but remain poorly characterized relative to fungal decomposers. Bacteria are generally more effective at decomposing easily simple carbohydrates in litter (Liu et al. 2022; Tláskal et al. 2016). Furthermore, some bacteria complement and facilitate fungal activity during decomposition when fungi are under

stress (Purahong, 2016; Berg and McClaugherty, 2014) and produce degradative enzymes in the final stages of litter disintegration (Kirby, 2005). To give an example, there are nitrogen-fixing bacteria (diazotrophs) that are capable of capturing nitrogen from the atmosphere (Burns and Hardy 1975). Diazotrophs interact with fungi when nitrogen availability is limited, thus helping the activity of lignolytic fungi (Hoppe et al. 2014). According to different studies, the main microbial groups involved in litter decomposition are: *Proteobacteria*, *Actinobacteria*, *Acidobacteria, and Bacteroidetes* (Liu et al. 2022; Baldrian *et al. 2012*; Tláskal et al. 2016). This underlines the relevance of microbes during decomposition and recycling of nutrients. Despite the importance of litter decomposition to understanding rates of CO_2 flux, the taxonomic identities and genomic capabilities of many key decomposer communities remain poorly understood.

CAZymes and litter decomposition

Bacteria and fungi can decompose leaf litter of different quality (Bradford et al. 2016). These microbial communities are the main producers of enzymes known as Carbohydrate Active Enzymes (CAZymes) giving them the potential to break down complex carbohydrates in plant cell walls (Guerreiro et al. 2023)**.** CAZymes are a diverse group of enzymes that play a relevant role in carbohydrate metabolism and are responsible for degrading compounds of plant litter, such as cellulose, hemicellulose, and lignin (Berg and McClaugherty 2014). The combined action of different microorganisms, along with their CAZymes is essential for nutrient cycling and decomposition (Zhang et al. 2022).

The main CAZyme families involved in litter decomposition include glycosyltransferases (GT) that synthesize carbohydrate chains, glycoside hydrolases

(GHs) break down cellulose and hemicellulose, carbohydrate esterases (CEs) remove esters to assist GHs, polysaccharide lyases (PLs) degrade uronic acid-containing polysaccharides, and auxiliary activity (AA) enzymes that enhance the effectiveness of other CAZymes in accessing carbohydrates. (Auer et al. 2023; Cantarel et al. 2009).

Extensive research has focused on the abiotic factors that drive leaf litter decomposition (Glassman et al. 2018), however, the structure of microbial communities, and the link between different species of trees and enzyme activities that modulate the decomposition process is still unknown (Guerreiro et al. 2023). While some studies have already analyzed the decomposition under different conditions, we still have a long way to go to understand this complex process. Since litter decomposition is affected by certain factors such as climate, type of litter, and enzyme activity, in the present study, we wanted to isolate and assemble bacterial genomes from leaf litter decomposition and analyze the functional capabilities of the isolates (such as CAzymes) and their putative roles in decomposition. Also, our goal was to investigate how a particular isolate is distributed across field sites. We hypothesized that there will be differences in the microbial community across different sites, and their ability to decompose the litter would be linked to the CAZymes activity.

Materials and Methods

Study sites

We used leaf litter samples that were previously collected from National Ecological Observatory Network forests (NEON) sites. These forest regions vary greatly in ecological and climatic traits, making these sites an ideal place for this research.

Three NEON sites shown in Figure 1 were selected to try to capture the microbial communities from colder and wetter sites located at higher elevations to hotter and drier sites at lower elevations. Bartlett Experimental Forest (BART) located in New Hampshire (Gamal-Eldin, 1998), Smithsonian Environmental Research Center (SERC) located in Maryland (Taylor et al. 2016), and Great Smoky Mountains National Park, Twin Creeks (GRSM) located in Tennessee (Smith, 2018).

Field experimental design

All the forests selected for study in the sites BART, SERC and GRSM are dominated by deciduous trees including red maple (*Acer rubrum*). We put 5 g of homogenized foliar fresh litter into nylon-membrane bags (Table 1). The bags with red maple leaves were deployed for the first time in the fall of 2021 (T0), then the samples were collected after 10 months in the summer 2022 (T1) to estimate the rates of decomposing organic matter, and other chemical traits. Additionally, litterbags were settled carefully at the same place from which each litter was sampled initially for upcoming analysis. All the litter samples were frozen, taken to the laboratory, and then stored at -80°C for further microbiological and molecular analysis.

Cultivation of litter bacteria

To isolate bacteria from leaves, we weighed 0.025 g of litter collected from the different NEON sites, and then we suspended the leaves of each sample in 1.0 ml of Ringer solution and shaken gently for 24 hours on an orbital shaker (125 rpm) at room temperature in a 1.5 ml Eppendorf tube under sterile conditions. Then, to have pure bacteria strains from leaves, we cultivated them using a conventional method and serial dilutions until 10^{-5} with Ringer solution, and 100 ml were added to agar plates with carboxymethylcellulose (CMC) and cycloheximide (Cycl) as a fungal inhibitor (Figure 2).

After 3-7 days, the resulting colonies were picked and subcultured until obtaining pure isolates. To identify them, the 16S gene was amplified with the Platinum II Hot Start PCR Master Mix (Invitrogen) using bacteria-specific primers 27F and 1492R (Ghyselinck et al. 2013) according to the producer's protocol. PCR conditions were initial denaturation at 94 °C for 2 min, followed by 35 cycles at 94 °C for 15 s, 60 °C for 30 s 68 °C for 1 min, and a final elongation at 78 °C for 10 min. PCR products were visualized by electrophoresis (1% agarose gel with the addition of SYBR Safe DNA gel stain and 1X TAE buffer).

Next, the amplification products were used to identify the taxonomy of each isolate through Sanger sequencing determining the nucleotide sequence of DNA fragments (Crossley et al. 2020). In this process the DNA is copied several times, and also includes chain-terminating nucleotides (dideoxy) which are framed by different colors. When amplifying the DNA, the polymerase enzyme will randomly add a terminator nucleotide therefore each end of the fragment that has been generated will have a unique color (fluorescent label) assigned to the last nucleotide. All the fragments

generated pass through a capillary electrophoresis gel in which the short fragments migrate quickly through the gel and the large fragments advance slowly. At the end of the tube, there is a laser that detects the pigment associated with each terminal nucleotide, allowing the reconstruction of the DNA sequence. Each detected fluorescence signal is recorded and read out on a chromatogram (Sanger and Coulson, 1975). Samples were sent for sequencing to Azenta. Following this, the sequences were analyzed, we used Basic Local Alignments Search Tool (BLAST) to identify the microorganism from each sequence obtained.

Genome sequencing of isolated strains

To assemble and annotate the bacterial genomes from leaf litter decomposition, first we sent to sequence at SeqCoast Genomics the whole genome of three isolates of interest for cellulose degradation (Table 2): L87a_D10^{-3}a_CMC_Cycl (Sample ID: L87a_a), L167_D10^{-4}_X2a_CMC_Cycl (Sample ID: L167_X2a), and L1168a_D10^{-4}_X19a_CMC_Cycl (Sample ID: L1168a_X19a) using Illumina NextSeq2000 platform, a 300 cycle flow cell kit to produce 2x150bp paired reads. Then, we used Knowledge Base (KBase), an open-source, collaborative biological data science platform developed by the U.S. Department of Energy (DOE) and has data available which includes 90,000 microbial genomes (Arkin et al. 2018).

Bioinformatics, genome assembly, and annotation

Before the assembly or alignment analysis, first we created a narrative entitled Assemble and Annotate Microbial Genomes. Once this narrative was completed, we uploaded the forward and reverse raw reads. Then, we used the different apps already developed in KBase, such as FASTQC to verify the quality of raw reads. Next, we used the app JGI RQC Filter to trim the read ends and remove bases with low quality. Subsequently, for quality control we used the PRINt SEQuences (PRINSEQ) which is a tool designed to filter out low-complexity sequences based on some criteria: GC content, ambiguous bases (Ns), and quality scores improving the quality of downstream analyses (Arkin et al. 2018; KBase, http://kbase.us).

After the read processing, we assembled genomic reads with the app metaSPAdes based on k-mers sizes and removed chimeric reads ensuring high-quality results. Then, we evaluated the quality and contamination of genomes with CHECK-M (KBase, http://kbase.us; Parks et al. 2015).

One of the tools for genome annotation used are Rapid Annotations using Subsystems Technology toolkit (RASTk) and PROKKA which are designed for bacterial and archaeal genomes and allow to annotate or assemble multiple genomes and prepare data to facilitate subsequent analysis. The Figure 3 shows the workflow to assign the taxonomy of bacterial genomes.

Relating isolate data to the dominant microbes detected from the field sites

We analyzed the 16S data bases (CSV files) previously generated. First, the metadata database contained the sample ID, the sites where the samples were collected, the species of tree selected, pH, precipitation and other abiotic factors.

Second, the mctoolsr database contained the OTU ID present in the different sites, and the corresponding taxonomy. All tests were carried out in R 3.6.2 (R Core Team 2019) using custom scripts and the mctoolsr, vegan, and ggplot2 packages (Left and Fierer, 2013). Table 6 shows the 20 most frequent operational taxonomic units (OTUs) present in leaf litter.

Results

Taxonomic characterization of putative cellulose-degrading bacteria isolated from leaf litter

We collected leaf litter samples from three sites across the eastern U.S. temperate forest and isolated 22 bacterial colonies after cultivation and restrikes to make sure we had pure colonies in CMC media and cyclohexamide. Table 2 shows a summary of the final samples that were processed. The dilutions factors from 10^{-3} to 10^{-5} were the most appropriate for obtaining pure isolates of microorganisms from the leaves of different samples and sites.

Once we isolated the different colonies from each sample and analyzed the 16S by Sanger sequencing method. After having the sequencing results, we identified in BLAST database the microorganisms that were present in the samples. Table 3 summarizes the isolates that were sequenced, and the third column shows the results provided by BLAST (BLAST hit).

We found the bacteria *Frondihabitans sp* in the isolates L87a_D10^{-3}a_CMC_Cycl (L87a_a) and L167_D10^{-4}–X2a_CMC_Cycl (L167_X2a). As well as the bacteria *Curtobacterium sp* present in two different isolates, one of them L1168a_D10^{4}_X19a_CMC_Cycl (L1168a_X19a) was analyzed. These microbes have

been isolated from various environmental sources, including leaf litter and maybe have a role in cellulose degradation (Zhang et al. 2007; Evseev et al. 2022). Therefore, we decided to sequence the whole genome of these samples.

The next columns in Table 3 (Sanger Length) shows the sequence length of read. The longest read lengths value was 1018 bp corresponding to the isolate *Burkholderia sp* (L87a_e), and for the isolates of our interest *Frondihabitans sp* (L87a_a and L167_X2a) had a length of 963 bp and 794 bp respectively. Additionally, *Curtobacterium sp* (L1168a_X19a) had a read length of 804 bp. Finally, we checked the Sanger quality values for each base in the sequence. Most of the values obtained were 40 or higher, confirming we had good sequence data.

Genomic characterization of *Frondihabitans*

In this study the complete genome sequences of the samples L87a_a, L1168a_X19a, and L167_X2a were analyzed in KBASE, and the results are shown in Table 4. After the read processing, genome assembly and annotation, GTDB-Tk - v2.3.2 assigned the taxonomy to each sample. L87a_a corresponded to the lactic acid bacteria *Levilactobacillus brevis,* L1168a_X19a matched with the genus *Enterobacter*, and L167_X2a sample correlated with the genus *Frondihabitans.* From now on we only focused on *Frondihabitans* isolate since the other samples seemed to be contaminated or not related to the cellulose degradation.

We also evaluated the completeness and contamination of the genome of *Frondihabitans*, and the CheckM analysis indicated a completeness of 99.49% and a contamination level of 1.68, confirming a high-quality genome assembly. According to QUAST tool, the final assembly of *Frondihabitans* resulted in 149 contigs with the

largest contig that is 1721360 bp in length. A total length of 4596069 reads were generated from Illumina NextSeq2000 platform (Illumina, USA). The annotation results in Table 5 indicate the *Frondihabitans* genome possesses mostly carbohydrate related genes. Notably, it has genes encoding for the functions of galactose, xylose, fructose, mannose, galactose, and sucrose degradation.

CAZymes genes in *Frondihabitans*

Specific CAZyme families associated with the microbial genome of *Frondihabitans* were detected. The DRAM app of KBASE generated a metabolism summary which provided a count of functional genes in a wide variety of metabolisms. For example, the gen GT2 for the family glycosyl transferases that encompass GT2 cellulose synthase; chitin synthase; N-acetylglucosaminyltransferase; hyaluronan synthase; chitin oligosaccharide synthase. The gen GH13 encodes Glycoside hydrolases such as GH13 alpha-amylase; cyclomaltodextrin glucanotransferase; cyclomaltodextrinase; trehalose-6-phosphate hydrolase; oligo-alpha-glucosidase; alpha-glucosidase; isoamylase; glucodextranase, and the gen AA3 which encodes CAZymes with auxiliary activities for instance AA3 cellobiose dehydrogenase; glucose 1-oxidase; aryl alcohol oxidase; alcohol oxidase; and pyranose oxidase. Additionally, the metabolism summary revealed that *Frondihabitans* exhibited a higher number of GTs (22), followed by GHs (12) and a lower number of AA (2) (Figure 4).

The Species tree app of KBASE constructed a species tree based on alignment similarity (Figure 5). Together with the GTDB-Tk - v2.3.2 tool and the assigned taxonomic classification (Figure 6), the outputs supported that the isolate L167_X2a corresponded to *Frondihabitants*.

Finally, the top 20 abundant bacterial OTUs in litter samples (Table 7) showed the top bacterial phyla found included Proteobacteria, Actinobacteriota and Verrucomicrobiota. A relevant finding was that *Frondihabitants* is on this list and *was* very abundant across 137 sites from our field samples.

Discussion

Our findings showed that temperate deciduous forests exhibited different bacterial taxa that influence the litter decomposition. A study by Newman et al. (2015) showed that fast breakdown leaf species such as red maple were more readily colonized by bacteria. In this research, we showed that one of the bacteria involved in decomposition, is *Frondihabitans* (Table 3 & 4). 16S rRNA gene and WGS analysis indicated that this bacterial isolate was a member of the phylum Actinomycetota (synonym Actinobacteria), class Actinomycetia, order Actinomicetales, family Microbacteriaceae (Figure 6). Actinobacteria play an important role in the bacterial community shifts that occur during leaf litter decomposition. They are present throughout the process from early to late stages of litter breakdown (Tláskal, et al. 2016) and have set of carbohydrate-active enzymes breaking down plant material, including cellulose and hemicellulose (Bao, et al. 2021).

Based on the available information in Table 7, *Frondihabitans* appears to have ecological and beneficial roles in litter decomposition. While *Frondihabitans* is not the most abundant microbe in litter environments, its abundance was high across 137 sites from the different field samples. Our results are consistent with the culture isolates study on the leaves by Purahong et al, 2016. They found that *Frigoribacterium* and

Sphingomonas (also members of the Actinobacteria phylum) were dominant bacterial genera initially present during the decomposition process. As a bacterium found in leaf litter, *Frondihabitans* likely acts as one of the early colonizers in the decomposition process and has an importance in nutrient cycling in forest environments. In fact, one of the species of *Frondihabitans* (*Frondihabitans australicus*) was originally isolated from fallen leaf litter from a pine forest located in Queensland, Australia (Zhang, et al. 2007). *Frondihabitans* is a Gram-positive bacterium, aerobic, non-spore-forming, irregular-shaped able to metabolize a range of carbohydrates, organic compounds and amino acids (Liu, 2014). Our analysis of isolates showed *Frondihabitans may* possesses enzymes involved in the biosynthesis and modification of complex carbohydrates found in plant litter and probably could break down plant material (Figure 4 & Table 6).

Our findings also showed the presence of genes related to carbon utilization, this is relevant because they have a key role in the degradation of different monosaccharides such as xylose, fructose, or galactose (Table 5). For instance, the activity of xylulokinase, breakdowns complex polysaccharides into xylose a simple sugar that can be further metabolized supporting the recycling of carbon and nutrients (Sciessere, et al. 2011).

We observed the presence of two of the major groups of CAZymes: Glycoside hydrolases and Glycosyltransferases (Figure 4 & Table 6). Glycosyltransferases transfer a monosaccharide to another molecule to form glycosidic bond (Vitthal-Gavande, et al. 2023). In this mechanism of reaction, the acceptor molecule attack at carbon-1 atom of a saccharide’s donor molecule (Rini, et al. 2022). This process is relevant since by modifying and synthesizing complex carbohydrates present in leaves

including cellulose, hemicellulose, therefore they become more accessible for degradation by other enzymes.

GT2 Cellulose synthase reported in Table 6 is a key enzyme responsible for cellulose biosynthesis. Cellulose synthase uses UDP-glucose as a substrate to polymerize glucose into linked glucan chains (Shundai, 2014). Glycosyltransferases work alongside glycosyl hydrolases enzymes that catalyze the hydrolysis of glycosidic bonds in complex carbohydrates like cellulose, hemicellulose (Vitthal-Gavande, et al. 2023; Linkins et al, 1990). Among the GH enzymes essential for cellulose degradation, we found the GH13 which is the major glycoside hydrolase family, GH13 contains α-Amylases which catalyze the hydrolysis of internal α-1,4-glucosidic linkages, also this family encompass hydrolases, and isomerases (Svensson, 1994). We also found GH16 endoglucanases such as Xyloglucanases (Table 6) which hydrolyze internal β-1,4-glycosidic bonds producing oligosaccharides; exposing and creating new chain ends which are essential for the subsequent action of exoglucanases and glucosidases (Datta, 2024).

Auxiliary activity enzymes are mainly redox enzymes involved in lignin breakdown and oxidative cleavage of polysaccharides, facilitating access for other CAZymes (Hobbs, et al. 2022). Cellobiose enzyme was identified in this study (Figure 4 & Table 6). This is a complex oxidoreductase which chemical reaction converts cellobiose into cellobiono-1,5-lactone and reduced the acceptor (Ouellette & Rawn, 2018). All these CAZymes may operate coordinately to break down complex compounds in leaves facilitating the recycling of carbon and nutrients in ecosystems.

Conclusion

We isolated several bacteria from leaf litter of *Acer rubrum* and one of the genomes assembled corresponded to *Frondihabitants*. After the genomic characterization of this microorganism, we found the presence of CAZymes glycosyl transferases, glycoside hydrolases, and auxiliary activity enzymes involved in the breakdown of complex carbohydrates. This suggests that *Frondihabitans* may play a role in carbohydrate metabolism, which could be important for litter decomposition. The potential ability of this isolate may function in degradation of the plant cell wall which is vital for nutrient cycling in ecosystems. Further research and detailed genomic analysis would be needed to identify and characterize unique CAZymes and features in *Frondihabitans*.

Future Directions

There are potential future research directions for studying *Frondihabitans* in litter decomposition. While our results do not elucidate specific contributions of *Frondihabitans*, they indicate that this bacterium could play important roles in decomposition through enzyme production. It would be valuable to study how *Frondihabitans* responds to climate change and altered environmental conditions. Additionally, research could explore and measure the enzyme production by *Frondihabitans* during different stages of decomposition. It could clarify the functional role of *Frondihabitans* in this process. Also, it would be promising to obtain more isolates and analyze them across the different sites and species of trees. The comparison of *Frondihabitans* activity across different litter types and sampling sites would be relevant to know the contributions *Frondihabitans* may make.

Tables and Figures

Table 1. NEON Sites and litter type chosen of the species of tree ACRU = red maple (*Acer rubrum*). Samples were collected 10 months after the litterbags were deployed by first time

SITE	ACRU Leaf sample
BART	X
GRSM	X
SERC	X

Table 2. List of samples cultured from different sites. It includes the site where we collected each sample (BART, GRSM, and SERC), the leaf litter selected (*Acer rubrum*), the sample ID with a code at the end of the name to identify the isolate that we picked, the isolate ID assigned to each colony including the dilution factor at which the organism grew, the time point to which the samples were collected (T1 = 10 months after the litterbags were deployed by first time in each site) and the plot selected within each sampling site.

Site	Tree Species	Sample ID	Isolate ID	Time Point	Plot
BART	ACRU	L167_a	L167_D10-^{5}a_CMC_Cycl	T1	21
BART	ACRU	L167_c	L167_D10^{-3}c_CMC_Cycl	T1	21
BART	ACRU	L167_d	L167_D10^{-3}d_CMC_Cycl	T1	21
BART	ACRU	L87a_a	L87a_D10^{-3}a_CMC_Cycl	T1	14
BART	ACRU	L87a_b	L87a_D10^{-3}b_CMC_Cycl	T1	14 14
BART	ACRU	L87a_e	L87a_D10^{-3}e_CMC_Cycl	T1	1
BART	ACRU	L1a_a	L1a_D10^{-5}a_CMC_Cycl	T1	1
BART	ACRU	L1a_b	L1a_D10^{-3}b_CMC_Cycl	T1	1
BART	ACRU	L1a_c	L1a_D10^{-3}c_CMC_Cycl	T1	1
BART	ACRU	L167_X1a	L167_D10^{-3}_X1a_CMC_Cycl	T1	21
BART	ACRU	L167_X2a	L167_D10^{-4}_X2a_CMC_Cycl	T1	21
BART	ACRU	L167_X3a	L167_D10^{-4}_X3a_CMC_Cycl	T1	21
BART	ACRU	L87a_X5a	L87a_D10^{-3}_X5a_CMC_Cycl	T1	14
GRSM	ACRU	L828_X7a	L828_D10^{-5}_X7a_CMC_Cycl	T1	22
GRSM	ACRU	L812_X10a	L812_D10^{-3}_X10a_CMC_Cycl	T1	19
GRSM	ACRU	L812_X11a	L812_D10^{-4}_X11a_CMC_Cycl	T1	19
GRSM	ACRU	L812_X12a	L812_D10^{-4}_X12a_CMC_Cycl	T1	19
SERC	ACRU	L1147a_X13a	L1147a_D10^{-4}_X13a_CMC_Cycl	T1	6
SERC	ACRU	L1193_X14a	L1193_D10^{-5}_X14a_CMC_Cycl	T1	11
SERC	ACRU	L1193_X15a	L1193_D10^{-5}_X15a_CMC_Cycl	T1	11
SERC	ACRU	L1270a_X17a	L1270a_D10^{-2}_X17a_CMC_Cyc	T1	25
SERC	ACRU	L1168a_X19a	L1168a_D10^{-4}_X19a_CMC_Cycl	T1	9

Table 3. Sanger sequencing results obtained from the different isolates cultured in CMC media + Cyclohexamide. Whole Genome Sequence of isolates studied are marked in bold.

Sample ID	Isolate ID	BLAST HIT	Sanger Length (bp)	Sanger Quality	WGS (Y/N)
L167_a	L167_D10-^{5}a_CMC_Cycl	*Robbsia andropogonis*	875	42	N
L167_c	L167_D10^{-3}c_CMC_Cycl	*Burkholderia sp*	950	47	N
L167_d	L167_D10^{-3}d_CMC_Cycl	*Caballeronia sp*	968	46	N
L87a_a	**L87a_D10^{-3}a_CMC_Cycl**	***Frondihabitans sp***	**963**	**47**	**Y**
L87a_b	L87a_D10^{-3}b_CMC_Cycl	Non Specific (Azenta result)	1	14	N
L87a_e	L87a_D10^{-3}e_CMC_Cycl	*Burkholderia sp*	1018	48	N
L1a_a	L1a_D10^{-5}a_CMC_Cycl	High background (Azenta result)	756	33	N
L1a_b	L1a_D10^{-3}b_CMC_Cycl	Sample did not amplify during PCR	NA	NA	N
L1a_c	L1a_D10^{-3}c_CMC_Cycl	Uncultured bacteria	958	48	N
L167_X1a	L167_D10^{-3}_X1a_CMC_Cycl	*Streptomyces sp*	810	50	N
L167_X2a	**L167_D10^{4}_X2a_CMC_Cycl**	***Frondihabitans sp***	**794**	**48**	**Y**
L167_X3a	L167_D10^{-4}_X3a_CMC_Cycl	*Non Specific (Azenta* result)	416	35	N
L87a_X5a	L87a_D10^{-3}_X5a_CMC_Cycl	*Paenibacillus taichungensis*	804	49	N
L828_X7a	L828_D10^{-5}_X7a_CMC_Cycl	*Acinetobacter higginsii*	784	49	N
L812_X10a	L812_D10^{-3}_X10a_CMC_Cycl	*Pseudomonadaceae bacterium*	779	49	N
L812_X11a	L812_D10^{-4}_X11a_CMC_Cycl	Non Specific (Azenta result)	66	18	N
L812_X12a	L812_D10^{-4}_X12a_CMC_Cycl	*Frondihabitans sucicola*	815	51	N
L1147a_X13a	L1147a_D10^{-4}_X13a_CMC_Cycl	*Chryseobacterium vietnamense*	816	49	N
L1193_X14a	L1193_D10^{-5}_X14a_CMC_Cycl	*Burkholderia sp*	804	51	N
L1193_X15a	L1193_D10^{-5}_X15a_CMC_Cycl	No significant similarity found	1	12	N

L1270a_X17a	L1270a_D10^{-2}_X17a_CMC_Cyc	*Curtobacterium sp*	814	51	N
9a	**L1168a_D10^{-4}_X19a_CMC_Cycl**	***Curtobacterium sp***	**804**	**50**	**Y**

Table 4. Summary of genome assembly of the three isolates submitted to whole genome sequence.

Sample ID	# Genomes	% Complete	% Contam	# Contigs	Largest Contigs	Total Length	Taxonomy
L87a_a	75	99.06	0.63	646	264141	1489780	*s_Levilactobacillus brevis*
L167_X2a	69	99.49	1.68	149	1721360	4596069	*g_Frondihabitans*
L1168a_X19a	223	99.49	0.08	15	268556	4314383	*g_Enterobacter*

Table 5. Genes related to carbon utilization as defined by Woodcroft et al., 2018 detected in the *Frondihabitans* isolate.

gene_id	gene_description	Module	Function	# of copies of the gene
K01785	aldose 1-epimerase	Galactose metabolism	galactose degradation	2
K00854	xylulokinase	Fructose and mannose metabolism	xylose degradation (isomerase pathway)	2
K00854	xylulokinase	Pentose and glucuronate interconversions	xylose degradation (oxidoreductase pathway)	2
K00078	dihydrodiol dehydrogenase / D-xylose 1 dehydrogenase (NADP)	Pentose and glucuronate interconversions	xylose degradation (weimburg/dahms)	2
K00847	fructokinase	Fructose and mannose metabolism	fructose degradation	1
K00849	galactokinase	Galactose metabolism	galactose degradation	1
K00965	UDPglucose-hexose-1-phosphate uridylyltransferase	Galactose metabolism	galactose degradation	1
K01784	UDP-glucose 4-epimerase	Galactose metabolism	galactose degradation	1
K01809	mannose-6-phosphate isomerase	Fructose and mannose metabolism	mannose degradation	1
K01187	alpha-glucosidase	Starch and sucrose metabolism	sucrose degradation	1
K01805	xylose isomerase	Fructose and mannose metabolism	xylose degradation (isomerase pathway)	1

Table 6. CAZymes families and genes found in the *Frondihabitans* isolate. The total number of CAZyme-encoding genes belonging to families known to degrade specific polysaccharides is shown in the last column.

Gene ID	gene_description	CAZyme family	Number of copies of the genes detected
GT2	GT2 cellulose synthase ; chitin synthase ; dolichyl-phosphate beta-D-mannosyltransferase; dolichyl-phosphate beta-glucosyltransferase; N-acetylglucosaminyltransferas; N-acetylgalactosaminyltransferase; hyaluronan synthase; chitin oligosaccharide synthase; beta-1,3-glucan synthase.	Glycosyl Transferases	22
GH13	GH13 alpha-amylase; cyclomaltodextrin glucanotransferase; cyclomaltodextrinase; trehalose-6-phosphate hydrolase; oligo-alpha-glucosidas; maltogenic amylase; neopullulanase; alpha-glucosidase; maltotetraose-forming alpha-amylase: isoamylase; glucodextranase; maltohexaose-forming alpha-amylase; maltotriose-forming alpha-amylase; branching enzyme; trehalose synthase; 4-alpha-glucanotransferase; maltopentaose-forming alpha-amylase; amino acid transporter	Glycoside Hydrolases	10
GH16	GH16 endo-1,3-beta-glucanase; xyloglucan:xyloglucosyltransferase; keratan-sulfate endo-1,4-beta-galactosidase; laminarinase; endo-1,3(4)-beta-glucanase; licheninase; xyloglucanase; hyaluronidase; endo-beta-1,4-galactosidase; beta-glycosidase; endo-beta-1,3-galactanase	Glycoside Hydrolases	2
AA3	AA3 cellobiose dehydrogenase; glucose 1-oxidase; aryl alcohol oxidase; alcohol oxidase; pyranose oxidase.	Auxiliary Activities	2

Table 7. The 20 most abundant operational taxonomic units (OTUs) observed in different leaf litter samples across sites and its taxonomic rank. *Frondihabitans* (highlighted in yellow) shown to be present in 137 sites.

Sample ID	Sample Present Across Sites	Kingdom	Phylum	Class	Order	Family	Gender
ASV_13	205	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	*NA*
ASV_12	191	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	*Bradyrhizobium*
ASV_8	173	Bacteria	Proteobacteria	Gammaproteobacteria	Burkholderiales	Burkholderiaceae	*Burkholderia-Caballeronia-Paraburkholderia*
ASV_1	168	Bacteria	Proteobacteria	Gammaproteobacteria	Burkholderiales	Burkholderiaceae	*Burkholderia-Caballeronia-Paraburkholderia*
ASV_6	168	Bacteria	Proteobacteria	Gammaproteobacteria	Burkholderiales	Burkholderiaceae	*Burkholderia-Caballeronia-Paraburkholderia*
ASV_48	151	Bacteria	Verrucomicrobiota	Verrucomicrobiae	Chthoniobacterales	Xiphinematobacteracea	*Candidatus Xiphinematoba*
ASV_4	142	Bacteria	Actinobacteriota	Actinobacteria	Micrococcales	Microbacteriaceae	*Galbitalea*
ASV_2	140	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Sphingomonas*
ASV_18	137	Bacteria	Actinobacteriota	Actinobacteria	Micrococcales	Microbacteriaceae	*Frondihabitans*
ASV_49	131	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	*Methylobacterium-Methylorubrum*
ASV_22	104	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	*Methylobacterium-Methylorubrum*
ASV_61	97	Bacteria	Verrucomicrobiota	Verrucomicrobiae	Chthoniobacterales	Chthoniobacteraceae	*Candidatus Udaeobacter*
ASV_28	86	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*
ASV_56	80	Bacteria	Verrucomicrobiota	Verrucomicrobiae	Chthoniobacterales	Chthoniobacteraceae	*Candidatus Udaeobacter*
ASV_86	78	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Sphingomonas*
ASV_3	72	Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	*NA*
ASV_27	66	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacterales	NA	*NA*
ASV_88	60	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	*Methylobacterium-Methylorubrum*
ASV_81	58	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	*Methylocella*
ASV_124	12	Bacteria	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	*Acidisoma*

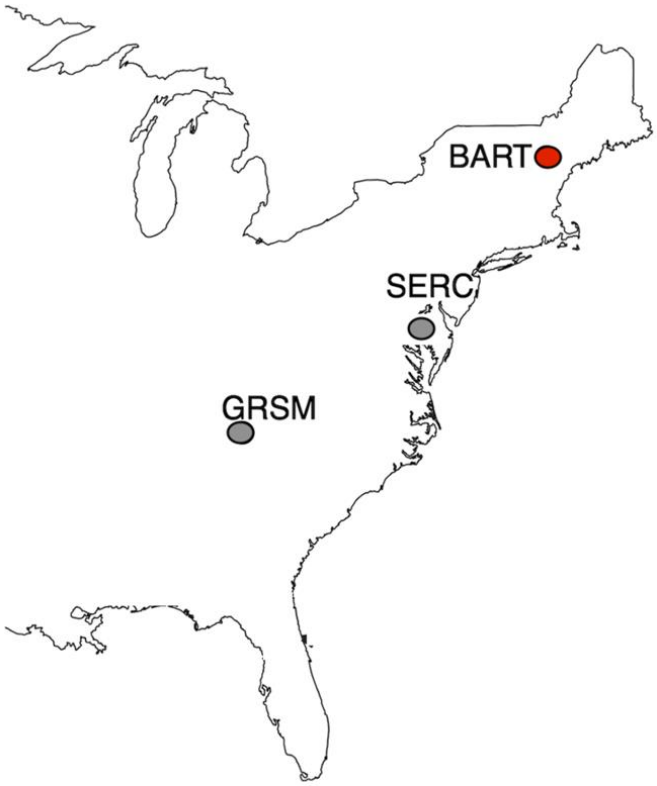

Figure 1. Map of the three NEON forest sites selected for litter decomposition sampling. Oliverio, A. For this project, we have selected one type of leaf litter (Acer rubrum) sampled from the sites BART, SERC and GRSM. The NEON site BART highlighted in red was selected for sequencing one of the genomes of interest (*Frondihabitans*).

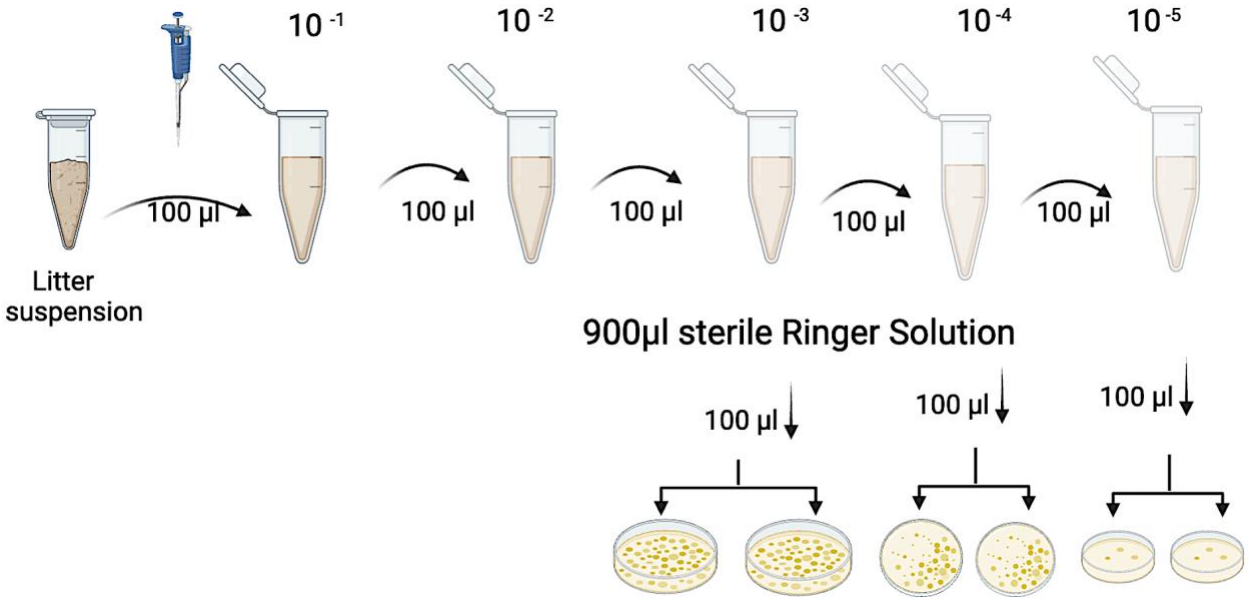

Figure 2. Isolation of leaf litter microorganisms. 100 ml of the original sample was taken and added to 900 ml of sterile Ringer solution. We made dilutions by a factor of 10. This process was repeated until the desired concentration was reached to obtain a pure culture of microorganisms plated in the culture medium CMC.

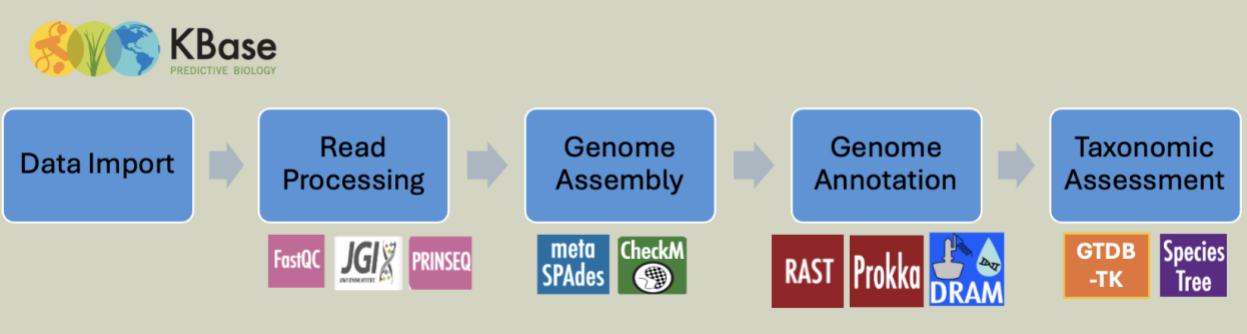

Figure 3. Workflow to assemble and annotate genomes using KBase tools. Modified from KBase, https://www.kbase.us/learn.

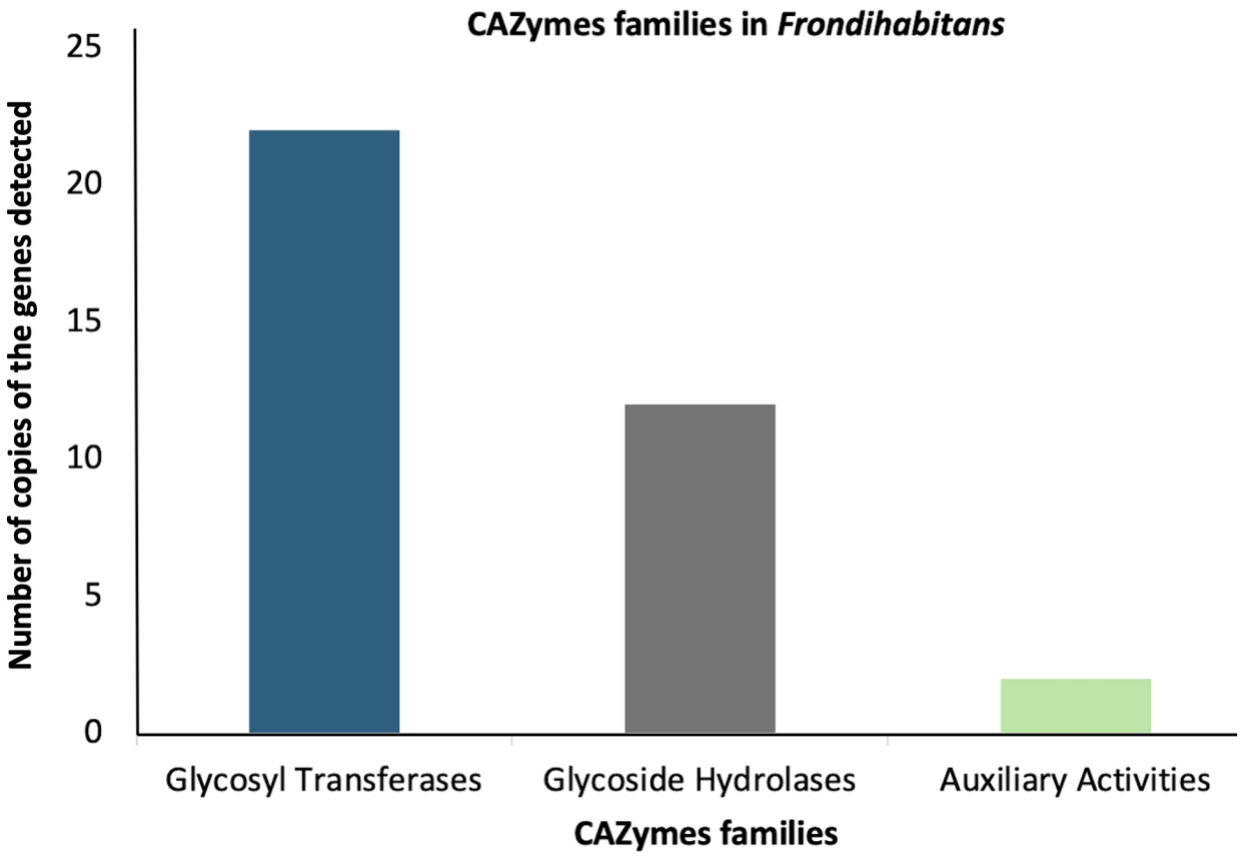

Figure 4. Genes related to CAZymes (output by DRAM annotations) that were detected in *Frondihabitans* and summarized by module.

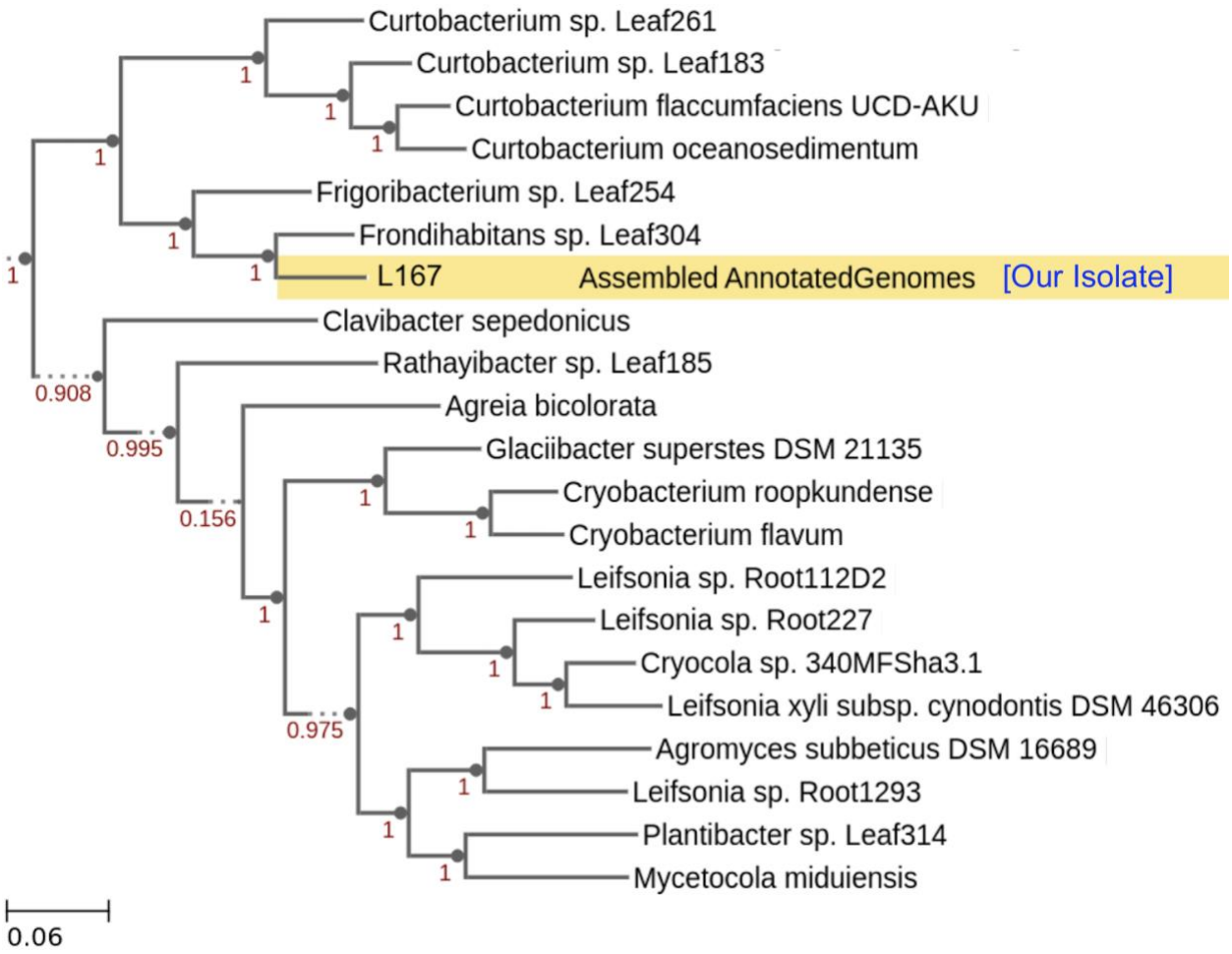

Figure 5. Species tree confirming isolate L167_X2a belongs to *Frondihabitans*. Numbers below the branches represent branch support bootstrap values.

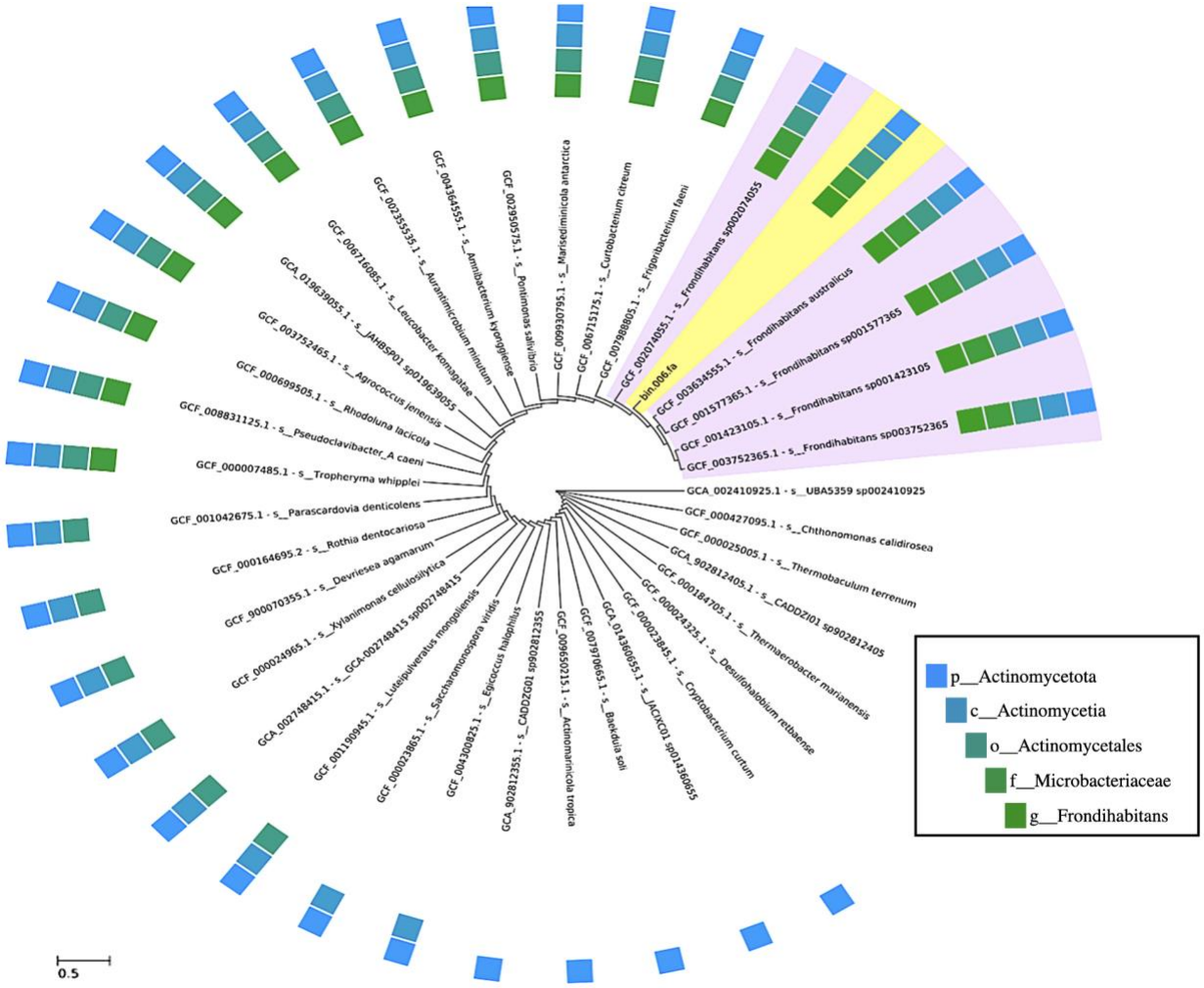

Figure 6. Assigned taxonomic classifications to bacterial genome of *Frondihabitans**.*** The sequences analyzed show high similarity to other species within the *Frondihabitans* genus.

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Vita

Olivia Rojo Nava

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EDUCATION

2022-2024 M.Sc. Biology, Syracuse University
College of Arts and Sciences, Syracuse, NY

2018-2020 M.Sc. in Biological Sciences, Evolutionary Biology.
Universidad Nacional Autónoma de México (UNAM)

2012-2016 B.S., Biology. Universidad Nacional Autónoma de México (UNAM)

2015 Pharmaceutical Sciences and Biology (International Student Mobility). University of Lausanne, Switzerland

PROFESSIONAL EXPERIENCE

2022 - 2024 Master of Science in Biology, Syracuse University
Cultivation and genomic analysis of litter decomposition isolates from eastern US temperate forests
Advisor: A. Oliverio

- Modified a protocol to isolate several bacteria from leaf litter of Acer rubrum. Assembled bacterial genomes from leaf litter decomposition using KBase and studied the functional capabilities of the isolates (such as CAZymes) and their roles in decomposition.

- Cultivated leaf litter material in plates, PCR tests, performed statistical and bioinformatic data analysis in R.

- Mentored 3 students during the winter 2022 and summer break 2023.

2023 - 2024 Teaching Assistant, Syracuse University
Molecular Biotechnology BIO 463-663 & Molecular Biology BIO 465-665

- Taught two lab sections (45 graduate and undergraduate students) per semester (2 semesters); prepare material for every lab experiment, requiring small class instruction, grading course materials and lab reports, developing new quizzes, mentor students as necessary, attending meetings with the principal professor of each class weekly.

2018 - 2020 Master of science. Institute of Biology – UNAM
Fungal diversity in native and hybrid corn assessed by metabarcoding
Advisor: A. Mastretta-Yanes

- Evaluated the landraces and hybrid maize fungus diversity in soil.

- Carried out DNA extraction from soil samples and mycorrhizal root tips, PCR, electrophoresis gels, and microscopy.
- Conducted field and greenhouse experiments.
- Lead one multidisciplinary team to keep communication and a relationship among farmers and researchers
- Interviewed farmers to know more about their agricultural practices and needs.
- Performed Statistical and bioinformatic data analysis in R and Excel.
- Designed the research protocol considering the resources, time and the budget granted.

2017 – 2018 Instructor. Facultad de Contaduría y Administración (FCA-UNAM)

- Taught Natural Resources Management and Sustainable Development to 90 undergraduate and graduate students and helped them to develop their Social Ventures.

2016 Research Assistant. Servicio Nacional de Sanidad Inocuidad y Calidad Agroalimentaria (Senasica).

- Developed a method to make *on-site* detections of *Listeria monocytogenes* in food samples avoiding false-positive results.
- Performed isolation and culture of microorganisms, biochemical tests, ELISA tests, designed oligonucleotides, and PCR tests.

2009 - 2015 Clinical Data Associate and Project Leader. INC Research

- Excelled in managing ~ 40 research protocols in different therapeutic areas
- Participated in the successful closure of several protocols and audits.
- Mentored 15 new entry staff.
- Implemented processes using macros in Excel for the management of databases, increasing productivity by 30% and ensuring the quality of data and protocols.
- Attended meetings and collaborated as the main point of contact among the project manager and other team members.

MEMBERSHIPS

2021 Society for the Study of Evolution, St. Louis, MO

2020 Mycological Society of America, Madison, WI

AWARDS

2022 Marilyn Sue Kerr Graduate Fellowship
2021 Opportunity 2022. Scholarship program from the U.S. Department of the State to pursue postgraduate education in the United States,
2018-2020 National Scholarship for Graduate Degrees in Mexico. Consejo Nacional de Ciencia y Tecnología (CONACYT)
2016 Bioinformatics Fellowship. Summer training to learn about Data Science.

2015	UNAM Student mobility. Fellowships offered by UNAM to students with outstanding academic records to study abroad.
2013	Pharmaceutical Star Award by INC Research. Employee performance recognition, acknowledging the hard work and accomplishments.
2012-2015	National Scholarship Program for Higher Education (PRONABES)

PUBLICATIONS

A. Urrutia, O. Rojo-Nava, C. Casas Saavedra, C. Zarate, Gustavo Magallanes, B. Hernández-Cruz and Oliver López-Corona (2021). A SDGs network analysis to enhance coherent evidence based policy: For SDG, the devil is in the interactions. Researchers.One, https://researchers.one/users/Oliver-Lopez-Corona/k5NGMGMY

Quantification of faecal matter as rediscovering source of nitrogen, phosphorus, and potassium in agrosystems (UNDER REVIEW)

MEETINGS, COURSES AND CONFERENCES

2023	STAMPS course. Strategies and Techniques for Analyzing Microbial Population Structures. (Marine Biological Laboratory)
2023	NEMPET. Syracuse University. *Poster presentation*
2021	Virtual Evolution Symposium. (Society for the Study of Evolution). *Speaker*
2020	Student Symposium of the Institute of Biology, UNAM,Mexico. *Speaker* https://www.youtube.com/watch?v=-7ZfEwpaBQk
2020	5th Mexican Population Genomics meeting, Mexico. *Poster presentation*
2020	The Mycological Society of America Virtual Conference (MSA 2020). *Poster presentation.*
2020	International Virtual Conference on Complex Systems. (ICCS 2020), Cambridge. *Poster presentation.*

VOLUNTEERING ACTIVITIES

2022 - Present	**Food Pantry Vineyard.** Serving food to over 300 families every weekend. Syracuse, N.Y.
2021	**Botanical Gardens Day.** Together with my advisor and labteam organized virtual workshops to publicize the tasks carried out in the Botanical Garden – UNAM.
2019	**Ocean Hackathon.** Worked with a multidisciplinary team and developed a prototype for the potential use of *Eichhornia crassipes*
2017 - 2020	**Volunteering with Emma Greenhouses Start-up**. Taught how to make your garden or your hydroponics system to grow your own food.
2013 – 2018	**Co-organizer of the TED Talks: TEDxMexicocity and TEDxIztapalapa**. Organized the event, performed fundraising, and coordinated the volunteer's crew.

2017 - 2022 **Co-founder Introduction to Bioinformatics course. FESI-UNAM**
Provide students with hands-on programming exercises with commonly used bioinformatics tools and databases.

2016 – 2022 Participated in the university's orientation days for new students. FESI-UNAM

LANGUAGES

Spanish Native
English Full professional proficiency. TOEFL IBT Score 87, Duolingo Score 125
French Intermediate working proficiency.

SKILLS

- Massive Sequencing of Bacterial Genomes
- Bioinformatic Analysis of Next-Generation Sequencing Data
- Intermediate proficiency in R and Python command line systems, basic statistical data analysis, and metabarcoding software (AMPtk, Phyloseq, mctools, DADA2)
- GitHub and Version control
- Estimation of total and dissolved C, N, and P concentrations in soil
- Creation, Development, and Direction of Social Enterprises
- Medidata – Rave, Oracle databases management
- Conducting and monitoring clinical research studies
- Human Development, Effective communication, Leadership, and Team building