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## Analysis of novel bacterial metagenome-assembled genomes from lignin-degrading microbial consortia

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### ABSTRACT

Despite recent progress, bacterial degradation of lignin is not completely understood. To address the mechanisms that bacteria from unknown taxonomic groups use to perform lignin-monomer degradation, functional analysis of bacterial metagenome-assembled genomes from soil-derived consortia enriched for microorganisms capable of degrading lignin was performed. A total of 232 metagenome-assembled genomes were recovered. After applying quality criteria of at least 70 % genome completeness and contamination less than or equal to 10 %, 39 genomes were obtained. From these, a total of 14 genomes from bacteria of unknown classification at lower taxonomic levels (i.e., only classified to the order level or higher) were chosen for further functional analysis. A global analysis of the potential ecological functions of these bacteria was performed, followed by a detailed analysis of monolignol degradation pathways. The phylum with the highest number of genomes was Proteobacteria. The genomes presented functions consistent with soil-derived bacteria, like denitrification, with different metabolic capacities related to the sulfur, chlorine, arsenic and carbon cycles, in addition to the degradation of plant cell wall components like cellulose, hemicellulose, and lignin. The Sphingomonadales\_OP 08 genome showed the greatest potential to degrade cellulose and hemicellulose, although it does not appear to be able to degrade lignin. The Actinobacteria\_BY 70 genome presented the highest number of enzymes and pathways related to the degradation of monolignols; furthermore, it showed the greatest potential for aromatic ring breakage by different fission pathways. The genomes of the two Actinobacteria showed the caffeic acid pathway, an important phenolic compound presenting several biological properties, such as antimicrobial and antioxidant. To our knowledge, this is the first time this pathway has been reported in this class of bacteria.

### 1. Introduction

Plant biomass is used in various types of bioprocesses as a feedstock. It is cheap, rich in carbon, easily available, and renewable. Plant-based biorefineries have emerged as an ecologically sustainable alternative to the oil industry, as biorefineries are capable of converting biomass into products ranging from electricity and biofuels to biopolymers (Santos and Magrini, 2018).

The three main components of plant biomass are cellulose, hemicellulose, and lignin. Lignin is the second most abundant polymer in nature, only behind cellulose. However, it is less studied, and there are

fewer industrial applications for it than for the other components of plant biomass. Chemically, it is an aromatic polymer composed of linked p-hydroxyphenyl (H), guaiacyl (G) and syringyl (S), derived from the monolignols coumaryl, coniferyl and sinapyl alcohols, respectively (Upton and Kasko, 2016). These monolignols are joined irregularly by ether bonds, giving rise to an amorphous three-dimensional molecule with functions of structural resistance, impermeability, and protection against pathogens (Ayyachamy et al., 2013). Degradation of lignin by enzymes is difficult due to its insoluble nature, and lack of easily hydrolysable bonds.

Bacterial degradation of lignin is still not fully elucidated, having

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lagged in comparison to knowledge about how fungi accomplish this. Understanding which bacteria play a role in this process and the metabolic pathways involved is an important initial step. Selection of specific microorganisms through enrichment protocols is a powerful way to obtain those that share a particular characteristic, such as the ability to use lignin as carbon source. Most enrichment protocols start with inoculation of liquid growth medium with a rich and diverse source of microorganisms like soil. After a certain amount of time in which the microorganisms most adapted to the growth conditions used multiply, an aliquot is transferred to fresh medium. This process is repeated several times until a stable community of selected microorganisms is obtained (DeAngelis et al., 2010). Thus, this method allows reduction in the complexity of a microbial community in a directed manner. While the enrichment process decreases the general and functional diversity, certain groups of microorganisms with similar biochemical functions become more abundant (Yu et al., 2015).

So far, only few studies have used such protocols to obtain microbial communities enriched for microorganisms able to use lignin as carbon source (Georgiadou et al., 2021; Mendes et al., 2021; Woo and Hazen, 2018; Z. Xu et al., 2018). In a study led by Xu et al. (2021), microbial community enrichment was used to investigate lignin degradation by bacteria. Rice straw was used as the only carbon source for a period of 7 days. The structure of the communities varied significantly throughout the different incubation phases, showing different levels of expression of the lignin degradation enzyme. The microbial consortium demonstrated effectiveness in degrading the lignin present in rice straw. In a study carried out by Díaz-García et al. (2020), three soil-derived microbial consortia were enriched in crops with “pre-digested” plant biomass. Sixty genes encoding enzymes involved in lignin catabolism were chosen, 20 of these genes were significantly abundant in the consortia, using metagenome analysis. These studies highlight the current status and advancements in understanding lignin degradation by microbial communities.

Metagenomics facilitates the bioprospecting of microorganisms with specific functional capabilities by allowing direct and comprehensive analysis of microbial communities from environmental samples, without the need for cultivation (C. Liu et al., 2021). This approach identifies microbial diversity, including rare or unculturable microorganisms, and reveals new genes and metabolic pathways, providing detailed functional mapping (Ferrer et al., 2016). Furthermore, metagenomics allows the analysis of interactions within microbial consortia. However, despite the depth of analysis that bioinformatics allows, it is important to consider that using this methodology may not be fully aligned with actual results from biological systems.

Here we present our work using enrichment-based metagenomics to investigate lignin biodegradation by soil-derived bacteria. Enrichment-based metagenomics can be used not only to identify the bacteria present in such communities, but also to investigate their ecological functions and metabolic capabilities. Focus was on the analysis of the second stage of lignin degradation, in which the monomers from lignin degradation enter enzymatic pathways that result in the breakdown of the aromatic ring. Importantly, molecules of high economic value, such as vanillin, ferulic acid, catechol and coniferol, are part of these pathways (Tramontina et al., 2020) (Brink et al., 2019).

## 2. Material and methods

### 2.1. Enriched microbial consortium for lignin use

As previously described in Mendes et al. (2021), three experiments were performed to obtain microbial consortia enriched for microorganisms able to use switchgrass lignin, extracted by the alkaline method (BE-Lig), at 30 °C as the carbon source. The alkaline method for lignin extraction consisted in grinding 12.5 g of biomass and adding to it 250 mL of 0.5 M NaOH. This was sterilized by autoclaving for 30 min at 121 °C. Subsequently, H<sub>2</sub>SO<sub>4</sub> 5 M was added until the pH of 7 was obtained.

The solution was kept for 24 h at 4 °C, and then centrifuged at 10,000 x g for 30 min at 4 °C. The supernatant was transferred to a second flask and centrifuged at 18,514 x g for 1 hour at 4 °C; and the new supernatant was sterilized with 0.45 µm pore filter. Briefly, the source of microorganisms for each of the three experiments, was backyard compost soil (BY) which was obtained from Alameda County (California, USA), Miracle Growth commercial soil (MG), and Organic Plus commercial soil (OP). Every 2 weeks, a subculture (called passage) was made by transferring an aliquot of the culture into fresh of M9 medium with trace elements, supplemented with 10 % (w/w) lignin extracted by the alkaline method. Over a 10-week period, 6 passages were performed for each of the three experiments. Samples from the third passage were used for metagenomic DNA (eDNA) extraction. eDNA extraction was performed with the All Prep DNA/RNA kit (QIAGEN), following the manufacturer's instructions.

### 2.2. eDNA sequencing and assembly of metagenome-assembled genomes (MAGs)

Paired-end sequencing (2 × 150 bp) of the eDNA was performed by the Joint Bioenergy Institute (JGI) (Walnut Creek, CA, USA) using the Miseq platform (Illumina). The generated sequences were filtered and superimposed using Bbtools version 36.69 (<http://bbtools.jgi.doe.gov>) (Bushnell et al., 2017). For the BY and OP samples, two runs were performed. Duplicates of each sample were combined and proceeded to assembly using MEGAHIT v1.0.6 (Wu et al., 2016) in standard mode, for the MG sample only one run was performed. The methodology flow to generate complete and selected genomes from metagenomes was based on Chen et al. (2019).

### 2.3. Taxonomic analysis of enriched microbial consortia

Taxonomic classification of all Metagenome-Assembled Genomes (MAGs) was performed with Kraken2 (Wood et al., 2019) against the standard database (Refseq), using the archaeal, bacterial, fungal, and plant libraries. The genomes of the samples were retrieved using the MaxBin2 v2.2.6 program (Chen et al., 2019; Wu et al., 2016) and selected with Ubin v0.9.14 (Bornemann et al., 2020; Chen et al., 2019). The completeness and contamination of genomes was estimated using CheckM v1.0.13 (Parks, 2014). A detailed metabolic analysis was only performed on recovered genomes that met specific quality (completeness greater than 70 %, and contamination less than or equal to 10 %), and novelty criteria (unknown classification according to Kraken2 in the lower taxonomic levels of genus and species).

### 2.4. Sequence alignment

Global sequence alignment was performed via the average nucleotide identity measure based on BLAST + (ANi) with the cut off 95 % for species, using the JSpeciesWS platform (<https://jspecies.ribohost.com/>) (Richter et al., 2016). The choice of database genomes was based on and performed by Tetra Correlation Search (TCS) estimations performed through JSpeciesWS. Sequence alignment was performed using MAFFT (Katoh and Standley, 2013) and construction of the phylogenetic tree via FastTree (Price et al., 2010), both performed using Geneious Prime 2023.2. Orthologous genes, annotated by PROKKA, present in the genomic core were used to construct the phylogenetic tree.

### 2.5. Profiling of microbial genomes for metabolic traits

The metabolic capacity of MAGs from the microbial communities enriched for lignin degradation was evaluated using METABOLIC v4.0 (Zhou et al., 2022), with the cut-off value of 75 % (i.e., having >75 % of the presence of MAG genes of a certain function). In addition, the profiles for HMMs (Hidden Markov models), Kofam, Pfam, and TIGRFAM were used; (Aramaki et al., 2020) to search for new genes of biotechnological interest, based on curated information on the eLignin database

([www.elignindatabase.com](http://www.elignindatabase.com)) (Brink et al., 2019). Using the data generated by METABOLIC, the global capacity of these genomes in relation to carbon cycle, arsenate metabolism, oxidative phosphorylation, nitrogen cycle, sulfur cycle, dehalogenation, and photosynthesis was also analyzed.

## 2.6. Annotation of metagenome-assembled genomes

Functional annotation of MAGs was performed with Rapid Prokaryotic Genome Annotation (PROKKA) v1.14.5 (Seemann, 2014). In addition, catalytic and carbohydrate-binding modules of enzymes that degrade, modify, or create glycosidic linkages were identified against the Carbohydrate-Active enZYmes database (CAZyDB 31–07–2020) (<http://www.cazy.org/>) using Diamond v0.9.22 with cutoff value  $<1E-102$ . The main metabolic pathways in lignin degradation were obtained from the eLignin database (<http://www.elignindatabase.com>) (Brink et al., 2019). Key enzymes of each reaction were compared with the annotation made by PROKKA for each selected MAG.

## 3. Results

### 3.1. Sequencing and assembly of MAGs

Altogether, for the samples of the 3 consortia (BY, MG and OP), 125,214,351 paired-end sequences were obtained (Table 1), and 1811,764 contigs were generated by metagenome assembly.

From the sequenced metagenomes for each of the consortia (BY, MG and OP), a total of 232 MAGs were recovered, including MAGs of fungi, bacteria and archaea origin, 39 of which were from bacteria and presented completeness greater than 70 %, contamination less than or equal to 10 % (Table 2). The largest portion of these genomes belongs to the phylum Proteobacteria, which was recovered in the 3 samples, BY (7), MG (5) and OP (8). Members of the phylum Bacteroidetes were also recovered in the 3 samples, BY (2), MG (3) and OP (7), while Actinobacteria was recovered only in BY (3) and OP (1). Quality genomes belonging to the phyla Acidobacteria (1), Chloroflexi (1) and Planctomycetes (1) were recovered only in the BY sample (Table 2). Only 2 MAGs were classified at the species level, one being *Bdellovibrio bacteriovorus* (Bdellovibrio\_OP 01) and the other *Klebsiella oxytoca* (Enterobacterales\_BY 56).

### 3.2. Description of MAGs

A total of 14 MAGs were chosen for metabolic profile analysis (Table 3). Selection of these MAGs was based on their unknown lower-level taxonomy; these MAGs were only classified to the order level or higher. The phylum with the highest number of representatives was Proteobacteria (8 MAGs), followed by Actinobacteria (2), Bacteroidetes (2), Chloroflexi and Acidobacteria with 1 MAG each. Regarding taxonomy of the 14 MAGs at class level, Alphaproteobacteria had the highest number of representatives (6 MAGs), followed by Gammaproteobacteria (3), Actinobacteria (2), Sphingobacteria and Acidobacteria, both with 1 MAG each, and 2 MAGs without taxonomic classification at this level. Finally, regarding the taxonomic level order, there were MAGs classified as Sphingomonadales (4 MAGs), Rhizobiales (1), Sphingobacteriales (1), Acidobacteriales (1) and Xanthomonadales (1), and MAGs without classification at this taxonomic level.

**Table 1**

Number of sequences, contigs and metagenomes-assembled genomes (MAGs) generated in the assembly of genomes in each consortium.

Consortium	BY	MG	OP	Total
Number of sequences	45,640,829	26,345,720	53,227,802	125,214,351
Number of contigs	795,053	439,984	576,727	1811,764
Number of MAGs	100	53	79	232

**Table 2**

Phylum-level taxonomic classification of the 39 Metagenome-Assembled genomes (MAGs) with  $>70$  % completeness and  $\leq 10$  % contamination recovered from the 3 microbial consortia initiated from BY, MG and OP soil samples and enriched for organisms capable of utilizing lignin extracted by the alkaline method (passage 3 at 30 °C).

Taxonomy	BY	MG	OP
Acidobacteria	1	0	0
Actinobacteria	3	0	1
Bacteroidetes	2	3	7
Chloroflexi	1	0	0
Planctomycetes	1	0	0
Proteobacteria	7	5	8
Total	15	8	16

When comparing the genomes of microorganisms assembled by metagenomic methodologies, it is observed that organisms of the same taxonomic class present similar genome sizes and CDS amounts (Table 4), due to shared evolutionary and functional characteristics. However, striking differences can arise when microorganisms come from distinct microbial consortia, as in the case of Gammaproteobacteria\_OP 27 and Proteobacteria\_BY 67. In contrast, organisms such as Alphaproteobacteria\_MG 62 show more comparable values (Table 4). Functional diversity and horizontal gene transfer are critical factors for these genomic discrepancies. MAGs within the same class and/or order were compared to determine if they were identical (Table 5). The average nucleotide identity based on BLAST + (ANiB) was used as a metric to evaluate the alignment of the 14 chosen MAGs. ANiB is defined as a pairwise measure of similarity between two sequences, in which the query genome sequence is fragmented, within the program itself, into smaller sequences, and these fragments are then compared against the other genomes to find homologous regions (Goris et al., 2007). In the case of this study, none of the MAGs compared are from the same bacterial species, since the ANiB was  $<95$  % in all cases. The phylogenetic analysis used for the tree construction was based on the genomic tetra search correlation (TCS) of the MAGs, shown in (Fig. 1). The comparison against type genomes showed differences between MAGs that share the same taxonomic classification. While Bacteroidetes\_MG 52 is closer to Chitinophaga, Bacteroidetes\_OP 02 is to Mucilagibacter. Actinobacteria\_BY 70 is phylogenetically closer to Illumabacter, while Actinobacteria\_2\_71 is to Rhabdotherrmincola. Unfortunately, it was not possible to determine the differences and which genomes are phylogenetically closest to the MAGs of Alphaproteobacteria.

### 3.3. Profile of metabolic traits

For all 14 selected MAGs, METABOLIC was used to identify the presence of general function metabolic pathways (Fig. 2). Most MAGs (79 % of them) showed some type of pathway involved in nitrogen metabolism. However, all identified reactions were related to nitrification, and none of these MAGs showed steps related to nitrogen fixation. A large part of the MAGs (71 % of them) also participates in the sulfur cycle, mainly in sulfur oxidation.

All MAGs from the 3 consortia encoded for enzymes that catalyze reactions within the carbon cycle: all these genomes are genetically capable of oxidizing carbon dioxide (Fig. 2). Only Actinobacteria\_BY 70 has genes related to rubisco carbon fixation. Furthermore, all representatives of MG and OP soils do acetic fermentation, while for BY-derived MAGs, only Actinobacteria\_BY\_2\_71 do not. In OP MAGs, there is one MAG with methanotrophic capability (Bacteroidetes\_OP 02). Three MAGs showed the presence of dehalogenation metabolism (Sphingomonadales\_OP 20, Bacteroidetes\_MG 52 and Proteobacteria\_BY 67). Most of the MAGs had arsenate reduction metabolism, being approximately 86 %. Meanwhile, 29 % of the MAGs showed some sort of chlorine metabolism.

MAGs showed different metabolic capabilities for amino acid

**Table 3**

Taxonomy of 14 Metagenome-Assembled Genomes that met the criteria of completeness above 70 % and contamination below 10 % and unknown lower levels of taxonomy.

Bin_ID	Completeness (%)	Contamination (%)	Taxonomy
Bacteroidetes_OP 02	99.82	1.61	k_Bacteria;p_Bacteroidetes
Sphingomonadales_OP 08	97.78	3.71	k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Sphingomonadales
Rhizobiales_OP 15	89.95	4.09	k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Rhizobiales
Sphingomonadales_OP 20	96.57	4.4	k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Sphingomonadales
Gammaproteobacteria_OP 27	91.99	2.49	k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria
Alphaproteobacteria_MG 62	79.33	0.43	k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria
Alphaproteobacteria_MG 3.66	84.94	1.75	k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria
Bacteroidetes_MG 52	73.71	1.72	k_Bacteria;p_Bacteroidetes;c_Sphingobacteria;o_Sphingobacteriales
Sphingopyxis_MG 67	86.49	4.44	k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Sphingomonadales
Acidobacteria_BY 58	83.15	2.95	k_Bacteria;p_Acidobacteria;c_Acidobacteria;o_Acidobacteriales
Actinobacteria_BY 70	97.44	4.8	k_Bacteria;p_Actinobacteria;c_Actinobacteria
Actinobacteria_BY 2.71	85.47	7.83	k_Bacteria;p_Actinobacteria;c_Actinobacteria
Bacteria_BY 59	81.13	3.25	k_Bacteria;p_Chloroflexi
Proteobacteria_BY 67	96.15	2.97	k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Xanthomonadales

**Table 4**

Number of nucleotide bases, number of CDS, number of contigs, number of tRNA/tmRNA/rRNA of MAGs.

Bin_ID	No. of bases	CDS	No. of contigs	No. of tRNA	No. of tmRNA	No. of rRNA
Bacteroidetes_OP 02	3612,635	3087	59	40	1	8
Sphingomonadales_OP 08	3376,306	3247	226	48	1	–
Rhizobiales_OP 15	3630,568	3062	310	49	1	1
Sphingomonadales_OP 20	3336,497	3176	133	45	1	–
Gammaproteobacteria_OP 27	5572,383	4677	57	52	1	3
Alphaproteobacteria_MG 62	2903,567	2734	32	31	–	–
Alphaproteobacteria_MG 3.66	2578,393	2484	490	34	1	1
Bacteroidetes_MG 52	2214,610	2326	671	20	–	–
Sphingopyxis_MG 67	3173,101	2890	315	44	1	1
Acidobacteria_BY 58	4677,113	4501	1387	39	–	4
Actinobacteria_BY 70	4998,236	4729	226	52	1	3
Actinobacteria_BY 2.71	3107,947	3013	378	47	1	1
Bacteria_BY 59	3478,031	3282	489	43	1	4
Proteobacteria_BY 67	3672,179	3320	83	51	1	1

synthesis. Shingopyxis\_MG 67 was the MAG with the largest number of genes related to amino acid synthesis (Fig. 2).

As shown in Fig. 3, the MAG Sphingomonadales\_OP 08 showed the highest potential for the degradation of plant cell wall components cellulose and hemicellulose, due to the greater number of genes encoding different degradation enzymes. Interestingly, the same MAG does not seem to have the potential for efficiently degrading monolignols.

### 3.4. Functional annotation of MAGs selected for lignin degradation analysis

The annotation performed by PROKKA generated a functional table of the genome for each of the MAGs. Each table has hundreds of product lines from different loci of the annotated genome. These tables can serve as a means for quickly looking up the existence of a specific known gene and its position in the genome.

Aiming to identify the presence of metabolic pathways for the degradation of aromatic compounds related to the degradation of lignin and its derivatives, fourteen MAGs were chosen for a detailed analysis to identify the presence of enzymes related to lignin degradation. The lignin degradation enzymes that were searched for were obtained from the eLign Microbial database (<http://www.elignindatabase.com>). From this database, a total of 55 enzymes related to the main degradation routes of lignin intermediates were used in our analysis, all of which were annotated and cataloged. In addition, 4 more enzymes from the MetaCyc database (<https://metacyc.org>) were used in our analysis to complement the lack of information on p-cresol metabolism, as the list of enzymes involved in this pathway in the eLign database was incomplete (supplementary Table 1). All these enzymes are associated with what is called the core of monolignols' degradation (i.e., enzyme-catalyzed

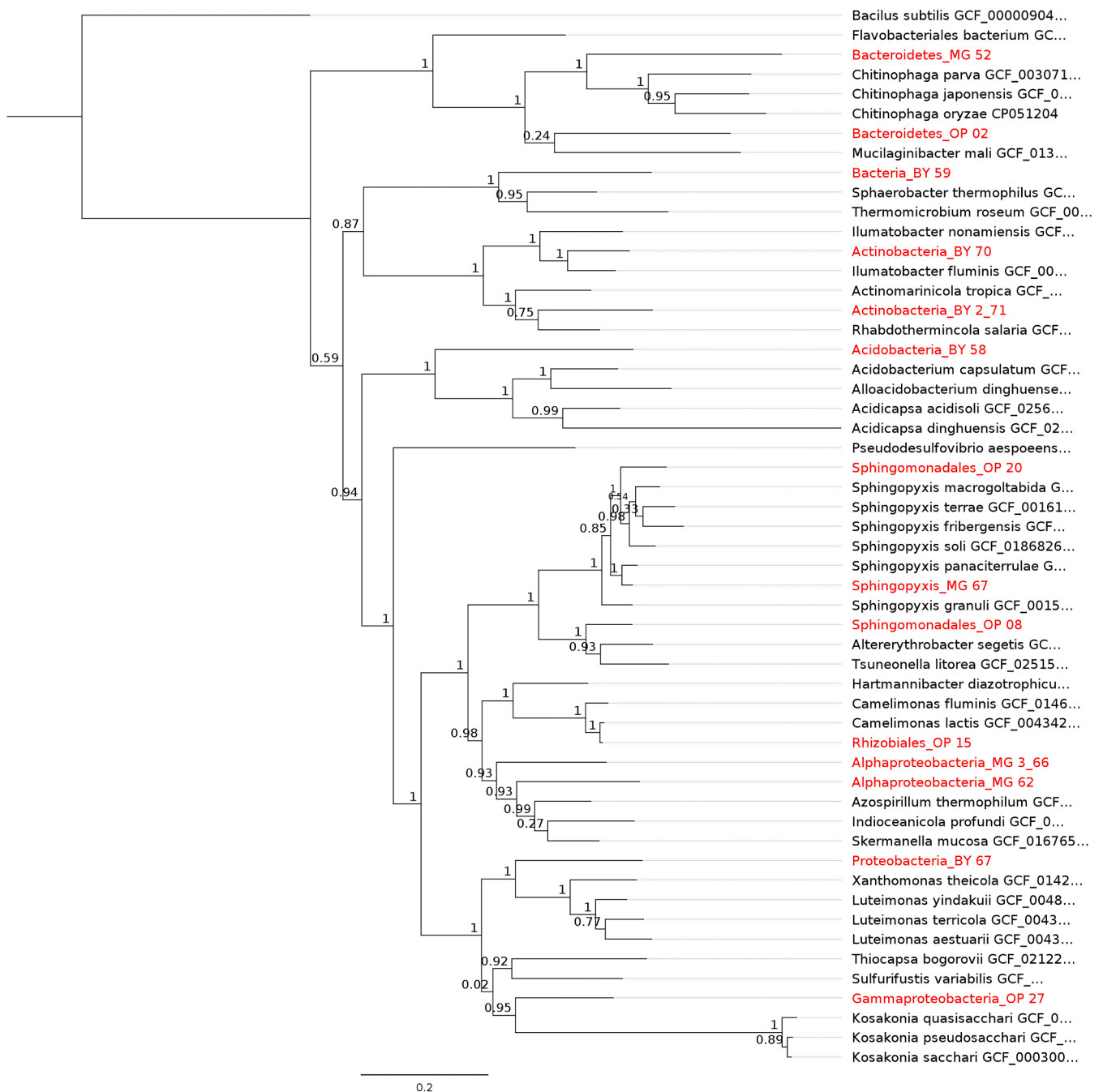
reactions that occur with lignin monomers). During the analysis, enzymes involved in lignin degradation pathways but not listed in the eLign Database were identified. This occurred because PROKKA, which annotates proteins in general, uses several databases for functional comparison, while for more focused analysis we chose the eLign database, which is specific for lignin monomers metabolism.

Annotation of the type of monolignol degradation enzymes present in each MAG, as well as the number of enzymes present, once again showed that the bacteria sharing the same taxonomic classification are different (supplementary Table 1). The MAGs Sphingopyxis\_MG 67 and Actinobacteria\_BY 70 showed the highest number of enzyme genes related to lignin degradation, both with 23 enzymes out of a total of 59 enzymes involved in the degradation of lignin monolignols (Table 6). For the reconstruction of the enzymatic pathways, the criterion used for a pathway to be considered as present was that 75 % of the pathway genes needed to be identified in the MAG (supplementary Table 1). During the metabolic reconstruction, it was possible to observe several incomplete pathways, shown by the small number of pathways, both for funneling and fission of the aromatic ring (Fig. 4).

Three main funneling pathways for monoaromatic catabolism have been defined, according to which of the main lignin units (or their derivatives), specifically the sinapyl branch (for S type), the coniferyl branch (for G type) and the p-coumaryl branch (for H type), are being catabolized.

For the coniferyl acid degradation pathway, only Sphingopyxis\_MG 67 and Sphingomonadales\_OP 20 showed the presence of at least 75 % of the enzymes involved (Fig. 4). Following the sequential reactions, none of the 14 MAGs have the enzymes needed for the ferulic acid degradation pathway; in the vanillin degradation pathway, only Actinobacteria\_BY 70 and Alphaproteobacteria\_MG 62 have the vanillin II pathway. As an alternative pathway for vanillic acid, the MAG





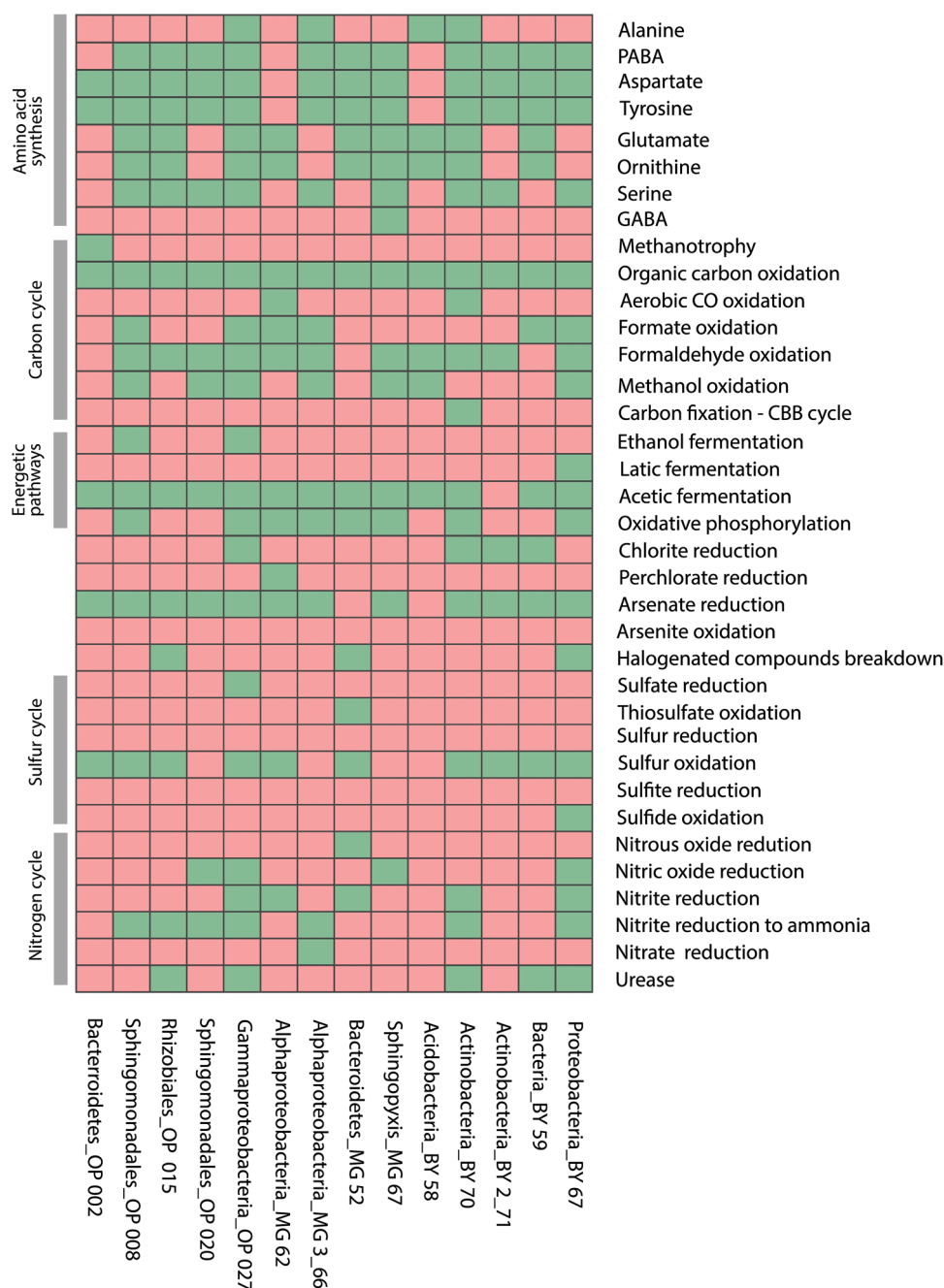
**Fig. 1.** Phylogenetic tree of 14 selected MAGs (in red). Sequence placement in the tree was based on 20 single-copy bacterial genes against type-genomes chosen based on nucleotide Tetra Correlation Search (TCS). The genes were manually concatenated and aligned by MAFFT. The evolutionary history was using the maximum-likelihood model based Generalized Time-Reversible (GTR) with bootstrap test. The horizontal bar at the bottom represents the percent number of changes in the evolutionary lineages.

148 h, except for vanillin, which was sampled only at 24 h, leading to a selection of fast-growing organisms as well (Levy-Booth et al., 2021).

An enrichment protocol to obtain microorganisms that are capable of metabolizing lignin has been used before (Georgiadou et al., 2021; Woo and Hazen, 2018; Z. Xu et al., 2018). In our previous work (Mendes et al., 2021), we have reported the bacterial diversity dynamics of the enrichment experiment in each passage, the consortia proved to be stable, in terms of bacterial diversity, from the third passage on (Mendes et al., 2021). There was a decrease in bacterial diversity, which is expected using this technique as selection occurs. Furthermore, in our previous experiment, the consortium originated from backyard soil (BY) showed greater initial bacterial richness and diversity when compared

to consortia derived from commercial soils (OP and MG); and this is probably due to the composting process, and because it did not undergo treatments for commercial sale (Mendes et al., 2021).

The study by Mendes et al. (2021) showed that the consortia derived from backyard compost soil exhibited greater initial richness and diversity compared to those from commercial soil. Additionally, a legacy effect was observed in the initial inoculum, as the higher bacterial richness and diversity in the backyard compost consortia persisted through the sixth passage, even after successive selection cycles. The data also highlights the impact of the carbon source on richness and diversity indices. Enrichment was conducted using kraft lignin and lignin extracted via the alkaline method. By the end of the enrichment



**Fig. 2.** Annotation of identified general metabolic pathways in MAGs using HMMS, Kofam, Pfam and TIGRFAM. Nitrogen cycle, sulfur cycle, dehalogenation, energetic metabolisms, arsenate, chlorine, carbon cycle and amino acids synthesis were annotated in the 14 chosen MAGs.

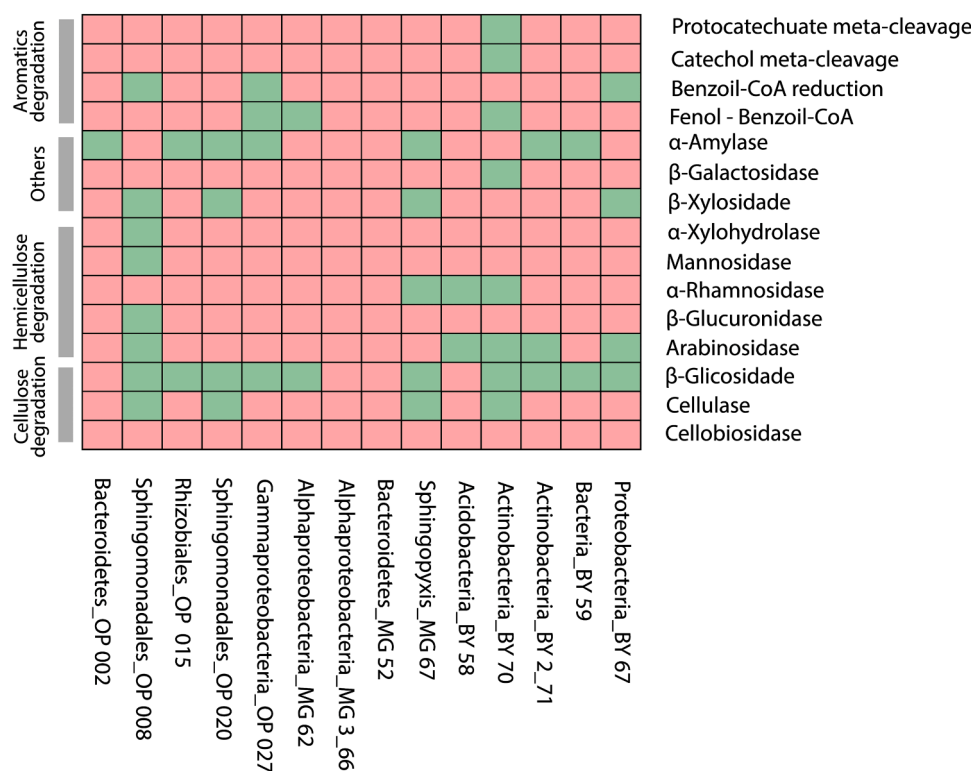
process, consortia utilizing lignin from the alkaline extraction method showed a higher number of operational taxonomic units (OTUs). Despite a reduction in richness and diversity after successive subculture cycles, the consortia remained highly complex. It can be inferred that the type of lignin used during enrichment was a key factor, as certain genera remained abundant only in consortia enriched with lignin from the alkaline method. Temperature was also analyzed, but the 7 °C variation between cultivation temperatures had less influence on the consortia than the type of carbon source (Mendes et al., 2021). Temperature, while important for microbial diversity, was not as significant in differentiating the consortia. In this present study, the choice of carbon source was due to the greater quantity of OTUs in the lignin consortia extracted by the alkaline method and the temperature of 30 °C was chosen, as there were no differences in diversity between the

temperatures tested.

#### 4.2. Taxonomic classification and description of MAGs

Molecular biology techniques together with bioinformatics tools are being used to identify taxonomic groups of microbial communities without the need for cultivation (Franco-Duarte et al., 2019; Sterflinger and Piñar, 2021). Due to the rich diversity of ecosystems related to the deconstruction of lignin and the complexity of this molecule, metagenomics has been applied in several types of studies. However, there are still a number of limiting factors for genome assembly, such as the degree of community complexity, and the technological process of assembly itself (Parks, 2014). Given these limitations, there are several studies reporting successful retrieval of high-quality population





**Fig. 3.** Annotation of the metabolic pathways related to the degradation of plant cell wall components, cellulose, hemicellulose and 4 of the main pathways related to the degradation of monolignols, in the 14 chosen MAGs.

**Table 6**

Total number of enzymes identified in each of the 14 chosen MAGs involved in the intracellular degradation of monolignols in the funneling pathways (enzymatic pathways that have an intermediate in common), fission pathways (enzymatic pathways in which the aromatic ring will be broken) and the count of the pathways present in each MAG.

MAGs	Number of enzymes	Number of funnel pathways	Number of fission pathways
Bacteroidetes_OP 02	7	1	0
Sphingomonadales_OP 08	18	1	1
Rhizobiales_OP 15	22	3	1
Sphingomonadales_OP 20	9	1	0
Gammaproteobacteria_OP 27	22	3	2
Alphaproteobacteria_MG 62	18	4	0
Alphaproteobacteria_MG 3_66	10	3	1
Bacteroidetes_MG 52	17	2	0
Sphingopyxis_MG 67	23	3	3
Acidobacteria_BY 58	13	1	0
Actinobacteria_BY 70	23	4	5
Actinobacteria_BY 2_71	8	3	0
Bacteria_BY 59	14	1	1
Proteobacteria_BY 67	17	1	1

genomes from metagenomic data (Albertsen et al., 2013; Sharon et al., 2013).

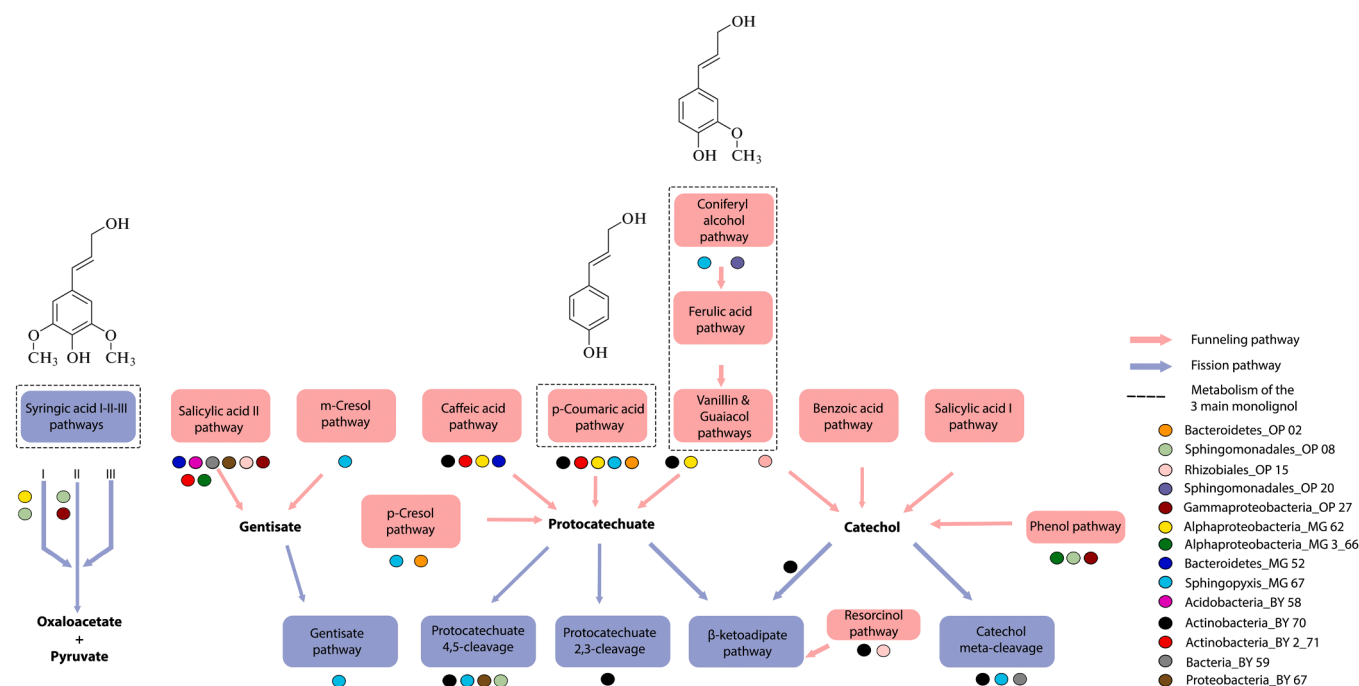
Metagenomics can identify and quantify the microbial diversity present in an environment, including non-culturable microorganisms (C. Liu et al., 2021). With advanced techniques and robust databases, it is possible to identify and resolve microorganisms down to the species level, as in the case of this study, in which it was possible to assign 2 MAGs to species (i.e., *Bdellovibrio bacteriovorus* and *Klebsiella oxytoca*). The taxonomic characterization of communities can allow studying microbial dynamics over time or in response to environmental changes.

Despite these capabilities, metagenomics is limited by difficulties in taxonomic resolution of microorganisms, as the accuracy of taxonomic classification depends on the comprehensiveness and quality of reference databases. Furthermore, the presence of environmental DNA or contaminants can complicate analysis (Chen et al., 2022). In addition, many organisms identified by metagenomics are still not culturable which may hinder practical applications of the microorganisms.

In a study carried out by Levy-Booth et al. (2021), a metagenome library was assembled with shotgun sequences from 351 different genomes from microbial consortia enriched with lignin from hot spring (30–45 °C) and mesophilic (17 °C) sediments from a park in Canada called Liard River Hot Springs. After quality filtering of these genomes, 39 genomes were obtained. The metagenome data was used to obtain taxonomic information on these genomes. Furthermore, the analysis showed metabolism consistent with oligotrophy, including pathways for the degradation of aromatic compounds such as syringate, vanillin, p-hydroxybenzoate and phenol.

The quality of metagenomic-assembled genomes can be evaluated based on the presence and absence of universal single-copy “marker” genes to estimate the integrity of each genome (Sharon et al., 2013). The completeness of a genome indicates how “complete” it is in terms of representing the expected genes for a specific lineage. While contamination in a genome refers to the presence of unrelated sequences, which can occur, for example, due to assembly errors. Choosing genomes with greater completeness and less contamination is essential to obtain more accurate conclusions. In the present study, the program used to evaluate the quality of assembled MAGs was CheckM. This program estimates the integrity/completeness and contamination of a genome using marker genes that are specific to a lineage, the so-called orthologous genes. In other words, the sets of lineage-specific markers used by the program provide robust estimates of lineages, with low estimates of completeness errors and genome contamination.

In the present study of microbial consortia selected for lignin utilization, we were able to recover a total of 232 genomes, and, of these,



**Fig. 4.** Lignin monomers degradation pathways for the 14 chosen MAGs (each shown as different colored circles according to the legend on the right side of the figure). The reactions represented by pink arrows are funneling reactions to an intermediate molecule (i.e., catechol, protocatechuate, gentisate, and 3-O-methylgalate in the syringic acid pathway), while the reaction represented by blue arrows are involved in the fission of aromatic rings. The routes of the primary monomers (S, H, G) are indicated by dashed boxes. Only MAGs that had at least 75 % of the genes for the pathway present were presented.

only 39 MAGs passed the cut off values calculated by CheckM of completeness greater than 70 %, and contamination less than or equal to 10 %. Of these 39 MAGs, only 2 MAGs were classified at the species level, one being *Bdellovibrio bacteriovorus* (BE\_OP.001) and the other *Klebsiella oxytoca* (LignBin\_BY\_Enterobacterales\_56\_11); both being gram-negative bacteria. In the literature, *Bdellovibrio bacteriovorus* has been described as an invasive predatory bacterium of other gram-negative bacteria (Sokkett, 2009), however, no relation to lignin degradation was reported. This suggests that the presence of this bacterium in this consortium may be due to its predatory character. Another possibility is that these bacteria can participate in biofilms that are rich in tryptone, "giving up" the predation of living cells, or even protecting the biofilm from exogenous bacteria (depending on the location of this species in the biofilm), when this biofilm is not rich in tryptone (Medina and Kadouri, 2009). On the other hand, *Klebsiella oxytoca* is known to be involved in the degradation of lignin. A least 262 genes associated with enzymes that degrade lignin have already been identified in the genome of this bacterium (dos Santos et al., 2020; Korsá et al., 2022).

From the 39 MAGs that met the quality criteria, we chose 14 to study that were classified only at high level taxonomic levels. This unsuccessful classification using existing databases, indicates that these 14 MAGs most likely represent new organisms, not previously sequenced, and deposited in public databases. These results also may help explain the presence of incomplete metabolic pathways in metabolism reconstruction.

When comparing the genomes of microorganisms assembled through metagenomic methodologies, it is often observed that organisms within the same taxonomic class exhibit similar genome sizes and number of CoDing Sequences (CDS). This similarity arises from shared evolutionary and functional traits of microorganisms within the same class, which are reflected in the fundamental genomic structure (Table 4). However, when microorganisms originate from distinct microbial consortia formed from highly divergent inocula—as seen in the present study, where three different types of soil were utilized—marked differences in genome size and CDS content can emerge, even among organisms belonging to the same class. This variability is exemplified in the

case of Gammaproteobacteria\_OP 27 and Proteobacteria\_BY 67, two Gammaproteobacteria displaying significant genomic divergence, likely due to their distinct consortium origin (Table 4). In contrast, organisms such as Alphaproteobacteria\_MG 62 and Alphaproteobacteria\_MG 3\_66 (Table 4) present more comparable values, reflecting their likely shared environmental pressures or functional roles.

These genomic discrepancies can be attributed to several factors. A critical factor is the functional diversity within the same taxonomic class, wherein microorganisms adapt to occupy different ecological niches (Baquero et al., 2021). This ecological differentiation can drive genome expansion or reduction, depending on the metabolic and environmental demands placed on the organism. Additionally, horizontal gene transfer (HGT) plays a significant role in increasing genome size in microorganisms, particularly when they need to acquire novel metabolic capabilities to thrive in symbiotic or competitive microbial consortia (Brito, 2021).

Despite these variations in genome size and CDS content, the presence of essential genetic elements, such as tRNAs, tmRNAs, and rRNAs, is generally conserved across microorganisms, regardless of genome size. These elements are crucial for the processes of translation and for maintaining the ribosomal machinery. However, the quantity and diversity of tRNAs may vary according to the specific adaptive requirements of the microorganism in its environment. Moreover, due to their relatively small size, these sequences may be lost or underrepresented during the metagenomic sequencing process, potentially contributing to the observed variation in their detection.

Table 7 summarizes previously published works, comparing different methodological approaches (16S rDNA and metagenomics) used to analyze microbial communities isolated from different sources. Enrichment practices vary, with some studies performing enrichment at regular intervals. The carbon sources used in enrichment show the diversity of organic substrates that can be analyzed for lignin degradation studies.

The predominant results reveal that Proteobacteria lineages are largely dominant in different conditions and types of enrichment, both for 16S rDNA and metagenomic studies. Lineages of Gammaproteobacteria, Alphaproteobacteria, Actinobacteria, and Firmicutes are also

**Table 7**

Comparison of microbial isolation, enrichment and carbon source methodologies from different environments.

Methodology	Origin of isolation	Enrichment	Carbon source	Predominant taxonomy	Reference
Metagenomic 16S rDNA	Soil Soil	Every 2 weeks Every 2 weeks	BE lignin Kraft lignin BE lignin	Proteobacteria Proteobacteria Firmicutes	This study (Mendes et al., 2021)
16S rDNA	Leaf samples	Every 24 h	Kraft lignin	Gammaproteobacteria Alphaproteobacteria	(Z. Xu et al. 2018)
Metagenomics	Sediments	0, 47, 96 and 148 h	Vanillin Kraft lignin	Gammaproteobacteria Alphaproteobacteria	(Levy-Booth et al. 2021)
Metagenomics	Compost	–	Compost	Actinobacteria Firmicutes Proteobacteria Bacteroidetes	(Ma et al., 2022)
Metagenomics 16S rDNA	Soil	–	Hemicellulose Cellulose Lignin DHP	Acidobacteria Actinobacteria Proteobacteria	(Wilhelm et al., 2019)
Metagenomics	Marine	Every 72 h	BE lignin	Proteobacteria	(Ley Yvette et al., 2023)

observed in different samples, suggesting a strong influence of the type of substrate and environmental conditions on the taxonomic profile of the communities. This highlights how the choice of methodology, isolation origin, and carbon sources affect the detected microbial diversity, with metagenomic methodologies allowing for greater detection of functional diversity. This could reflect the ability of each approach to capture taxonomic variation, including the presence of microorganisms with highly specialized metabolic niches.

#### 4.3. Metabolic profile

It is known that microorganisms are part of biochemical cycles mediating the transformation and cycling of elements. In nature, these microorganisms live in complex communities, and the metabolic interactions within these communities can be quite flexible. Several metabolic steps within a specific pathway are often distributed among various microorganisms. For example, in a study carried out by [Díaz-García et al. \(2020\)](#), in which community enrichment and metagenomics were used, it was possible to make the taxonomic affiliation of some genes linking specific functions with specific rates. Results suggest that lignin catabolism in these microbial systems is a specialized process in which each taxon has its own niche performing a specific job, such that one organism has the product of its reaction used as a substrate for another organism. A similar metabolic interdependence is also observed in Archaea, particularly Woesearchaeota, whose metabolic deficiencies necessitate survival within the context of consortia ([Liu et al., 2022, 2018](#); [X. Liu et al., 2021](#)). The reliance of Woesearchaeota on other organisms for survival suggests potential mutualistic or syntrophic relationships, and this dependence presents a significant challenge for their pure cultivation. Thus, strategies that encourage symbiosis could improve enrichment or isolation of these archaeal lineages, though more research is needed to fully understand their co-occurrence patterns in microbial communities.

The METABOLIC program was used in this study to evaluate the functional role of the microorganisms that make up the consortium enriched for those capable of lignin utilization in the context of biogeochemistry and metabolic networks at the community level. Considering the selected 14 MAGs, the consortia show different levels of metabolic capabilities within networks of biogeochemical cycles. >75 % of the MAGs showed some kind of relation with the nitrogen cycle, which is consistent with the fact that the microbial consortia originated from soil, where most of this cycle occurs. However, none of the consortia showed nitrogen fixation capacity, only denitrification capacity being present. The lack of genomes with nitrogen fixation capacity shows a disadvantage of the metagenomics methodology. Since this is a stable community and none of the 39 identified MAGs that passed the quality criteria also fix nitrogen, it is possible that within the other 193 MAGs assembled, but which were not selected due to the quality criteria

applied, there is an organism capable of nitrogen fixation. It is also possible that some MAGs may encode for genes involved in nitrogen fixation via some non-classical pathway not identified in the metabolism analysis.

The capability of dehalogenation, a chemical reaction in which an atom of a halogen (i.e., chemical elements that make up family 17 in the periodic table, the most common being fluorine, chlorine, bromine and iodine) is replaced by another, was observed in some representatives of the consortia. Although lignin degradation by microorganisms is not directly associated with dehalogenation, in genetic terms this activity can be confused with other biochemical functions, since the gene clusters for enzymes of the degradation steps of halogenated aromatics are similar to those which metabolize general aromatic (such as lignin) and non-halogenated compounds ([Pimviriyakul et al., 2020](#)). To verify this activity, it would be necessary to specifically test these organisms for this function.

Although arsenic does not have a metabolic role, nor is it a necessary nutrient for microorganisms, it is naturally found in rocks and sediments, and it can be naturally released into the soil ([Huang, 2014](#)), with no specific pathway identified for its absorption. Results indicate that most of the 14 MAGs showed arsenate reduction function. Arsenic uptake appears to occur using existing transport systems, most likely phosphate. This unspecific uptake of arsenate by microorganisms may simply be followed by efflux of arsenate from the cell, or the arsenate is rapidly reduced to arsenite, causing detoxification ([Huang, 2014](#)). Arsenate oxidation is also a way to decrease arsenate detoxification, but it is dependent on cellular respiration.

The fact that there are MAGs that appear to be unrelated to lignin degradation, such as the species of *Bdellovibrio bacteriovorus*, is expected because they are part of a microbial consortium. In a consortium, groups of microorganisms act together for a given action, presenting a complex activity network of interconnected functions. In these networks, the efficiency of ligninolytic bacterial metabolism emerges from the synergistic action of the main enzymes ([dos Santos et al., 2020](#)) from different organisms.

#### 4.4. Lignin degradation analysis

Lignin recovery is essential to increase sustainability and economic viability in biorefinery processes, although lignin decomposition and degradation are complicated by its structural recalcitrance. Both bacteria and fungi play significant roles in this process, often working together in microbial communities to efficiently break down lignin.

Fungi have been extensively studied for lignin bioconversion due to their ability to produce potent ligninolytic enzymes. However, due to the advantages that bacteria offer, these are beginning to be investigated. Some of the advantages of bacteria over fungi are the greater stability of bacterial cultures to extreme conditions of temperature and

pH, greater specificity of the biochemical reactions carried out by bacterial enzymes, in addition to greater ease of genetic manipulation when compared to fungi (Aarti et al., 2015; Huang et al., 2013; R. Xu et al., 2018).

The focus of this work was monolignols, the building blocks of lignin, because a detailed understanding of their structure and degradation can lead to significant advancements in the efficiency of the bioconversion process. We performed the annotation of predicted pathways for their degradation/ conversion into other molecules. By investigating how bacteria and fungi degrade these compounds, more effective methods can be developed for utilizing lignin as a valuable resource, thereby contributing to the sustainability of industrial biorefinery processes.

Several molecules of economic interest can be obtained from the degradation of lignin. For example, coniferol is typically used to synthesize various value-added chemicals such as pinoresinol (hypoglycemic agent), sesamin (anticholesterolemic and antihypertensive), and silybinin (hepatoprotective and used in the treatment of cancer and arthritis) (Tramontina et al., 2020)

The biotechnological production of coniferol, with the use of microbial-derived enzymes from low-cost agro-industrial residues, is a promising alternative for the supply of this compound. In a study carried out by Tramontina et al. (2020), the biocatalytic production of coniferol was observed, directly from lignocellulosic biomass, showing efficient conversion from ferulic acid. A biodegradation-biotransformation process is represented in this system, offering the potential to add value to agro-industrial waste. This is an unprecedented strategy to produce high-value fine chemicals within the context of a plant biomass biorefinery.

Lignin-derived aromatics can also be metabolized through a  $\beta$ -ketoacid pathway to synthesize polyhydroxyalkanoates (PHAs) in bacteria. PHAs are very promising bioplastics, as they have physico-chemical properties similar to those of plastics based on fossil fuels, in addition to having high biocompatibility and biodegradability, and can be applied as biomaterials in medical applications (Tan et al., 2021). In one study (Lin et al., 2016), a modified *Pseudomonas putida* consumed Kraft lignin and produced PHA at a titer of 73 % by dry weight of cells.

The lignin degradation literature is quite rich on physiological characterization of different organisms using specific aromatic substrates (Brink et al., 2019), without presenting intercellular conversion mechanisms or reporting evidence of a specific funneling pathway. In contrast, the molecular biology of specific metabolic pathways is much less known. However, knowledge of lignin degradation metabolic pathways is extremely important for the valorization of lignin by techniques such as advanced metabolic engineering and genetic engineering. The present work attempts to help fill this gap.

Some contributions that metagenomics work allows are functional annotation of genes through comparison with databases of known sequences; the discovery of new and rare genes that are not present in databases; identification of complete or partial metabolic pathways from metagenomic data; identification of metabolic interactions, and potential for manipulating metabolic pathways for biotechnology and bioremediation. Despite this, not all genes can be annotated accurately due to the lack of known homologies, presence of functional ambiguity and errors in gene function prediction. In addition, assembling complete genomes or metabolic pathways can be difficult due to sequence fragmentation. Therefore, predicting the actual functionality of metabolic pathways from metagenomic data can be challenging without experimental validation (Chen et al., 2022).

Our annotation for genes related to lignin degradation was based on PROKKA, and it occurred in two stages - one identifying the position of the gene, and the other comparing the gene to a database of known sequences at protein level and, consequently, their functions. If no protein match was found, it is annotated as "hypothetical protein". This annotation generated a functional table that, in this case, was used to assemble a degradation map of lignin monomers based on Brink et al. (2019), using the eLignin database. It is important to emphasize that

since information related to lignin-monomer degradation pathways found in different types of bacteria is scarce, including within the eLignin database, this may have contributed to the absence of several enzyme-coding genes that were annotated as hypothetical proteins.

The eLignin database (<http://www.elignindatabase.com/>) is a curated database. It contains information on organisms involved in lignin degradation, place of organism isolation (e.g., soil, water, sediments, termite gut, pulp), enzymatic pathways involved in lignin degradation, as well as other information (Brink et al., 2019). The metabolic routes present in this database are those of the second part of lignin degradation, in which the reactions are intracellular, and the aromatic rings are broken.

Based on the pathways identified by eLignin, it is evident that the 14 MAGs studied differ in their abilities to degrade lignin monomers. Lignins extracted from grasses contain a higher proportion of units derived from p-coumaryl alcohol (H) than other types of lignin (Silva et al., 2021), which may have driven the selection of organisms that can degrade this monolignol. Specialization in the degradation of specific monolignols is common, as different species of microorganisms tend to have enzymes that work on specific monomers. In other words, if an organism is capable of carrying out the p-coumaric acid degradation pathway, it will not necessarily be able to degrade coniferyl acid (Brink et al., 2019). However, there is the possibility of loss of information during genome assembly from the metagenome, because metagenomes have protein sequences lacking annotation or of unknown function (referred to as ORFans).

It is interesting to note that until now, within eLignin and in the literature in general, there have been no reports relating the Actinobacteria phylum to the degradation of caffeic acid, even though this is the second phylum with the greatest abundance of data within the database. Caffeic acid is a phenolic molecule produced through the secondary metabolism of plants, an important molecule for the synthesis of lignin, being the main hydroxycinnamic acid in human diet (Esp ndola et al., 2019). Several physiological effects of caffeic acid have been reported, such as antibacterial, antiviral, antioxidant, anti-inflammatory, anti-atherosclerotic, and anticancer activities (Esp ndola et al., 2019). In addition to caffeic acid being an important component of the lignin polymer, its enzymatic pathway of intracellular degradation generates protocatechuate, one of the main intermediates of the second stage of lignin degradation (Brink et al., 2019). Thus, the identification of genes related to the degradation of caffeic acid in 2 Actinobacteria MAGs (Actinobacteria BY 70\_11 and 2\_71\_9) is surprising and should be focus of future work. This finding is unlikely to be due to metagenome assembly errors, as the caffeic acid pathway genes were annotated in high quality MAGs presenting low contamination and high completeness, especially in the case of Actinobacteria BY 70 (i.e., completeness of 97.44 % and contamination of 4.8 %).

None of the 14 selected MAGs seem able to degrade lignin monomers effectively. This is because they lack several of the needed pathways, like the coniferyl alcohol pathway to the vanillin pathway. It is also important to note that while there are many MAGs with the caffeic acid and p-coumaric acid pathways, there are few MAGs that show the aromatic ring fission pathways from protocatechuate. This might be expected from bacteria because, when acting alone, they degrade lignin slowly or fail to degrade it efficiently. Therefore, for its degradation to occur, it is generally preferable to subject a lignin substrate to a mixture of bacterial species rather than a single one (Aarti et al., 2015).

## 5. Conclusions

Lignin recovery is essential for the sustainability and economic viability of plant biomass-based biorefineries. However, lignin decomposition and degradation are complicated by its structural recalcitrance. Knowledge on how bacteria process and bioconvert lignin has lagged behind that about fungi. In the present study, the taxonomy and general metabolic capabilities of three lignin-degrading microbial consortia

were analyzed. For this, 232 genomes were assembled from metagenomes of the three consortia, 39 of which showed high quality (high completeness and low contamination) and thus were selected for detailed analysis. Among these, one MAG stood out for its potential in cellulose and hemicellulose degradation, while another showed the highest number of genes related to monolignol degradation pathways, especially aromatic ring fission pathways. Importantly, the metabolic analysis uncovered for the first time that Actinobacteria have the genetic potential to degrade caffeic acid.

This work was as an initial step to uncover lignin degradation pathways in previously unknown bacteria using metagenomics. However, it is crucial to consider that metagenomics investigations are speculative and must be validated by classical microbiology and biochemistry methods, such as bacterial cultivation and enzymatic tests, to confirm the actual functionality of the identified genes and pathways. Despite these limitations, our metagenome analysis reveals the presence of genes associated with key enzymes involved in the lignin degradation process. The identification of these genes, in different members of the microbial consortia analyzed, is consistent with the ability to utilize lignin monomers as a source of carbon and energy.

The integration of *in silico* bioinformatics and experimental approaches is essential to obtain a complete and accurate understanding of the functional capabilities of microorganisms. More studies focusing on enzymatic pathways for degradation of lignin by bacteria will further contribute to its valorization, as these may reveal how molecules of economic interest are produced. Another area that should be further investigated in the future is genomic complementarity - specifically identifying and characterizing genes in different organisms that, when combined, can improve lignin conversion and degradation. Identifying interactions between different genetic components should give insights into how different organisms coordinate lignin degradation in the context of a consortium. Finally, future work should also focus on characterizing the biochemical functions predicted by bioinformatics for these enzymes of biotechnological interest.

#### Ethical statement

N/A

#### Credit authorship contribution statement

**Vitória Balestrini:** methodology, formal analysis, writing. **Otávio Pinto:** methodology, formal analysis. **Blake Simmons:** funding acquisition. **John Gladden:** conceptualization, funding acquisition, writing – review & editing. **Ricardo Krüger:** writing – review & editing. **Betania Quirino:** conceptualization, investigation, funding acquisition, writing – review & editing.

#### Data

The data is publicly available at the NCBI database. The BioProjects accessions numbers are: PRJNA444402 (BioSample accessions: SAMN41779481, SAMN41779482, SAMN41779483, SAMN41779484, SAMN41779485); PRJNA444403 (BioSample accessions: SAMN41789062, SAMN41789063, SAMN41789064, SAMN41789065, SAMN41789066) and PRJNA465151 (BioSample accessions: SAMN41788633, SAMN41788634, SAMN41788635, SAMN41788636).

#### Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Betania Quirino reports financial support was provided by Brazilian Agricultural Research Corporation. Betania Quirino reports financial support was provided by national research council of Brazil (CNPQ). If there are other authors, they declare that they have no known

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#### Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.crmicr.2024.100302](https://doi.org/10.1016/j.crmicr.2024.100302).

#### Data availability

Accession numbers are provided in this version of manuscript.

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