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Catabolic potential of bacteria isolated from a contaminated environment: a genomic approach on aromatic hydrocarbon degradation

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Abstract

The constant demand for petroleum and the recurrence of environmental accidents have spurred the development of sustainable remediation strategies, among which bioremediation stands out for its efficiency and low environmental impact. In this study, a functional genomic analysis was conducted on a microbial consortium composed of nine bacterial strains isolated from a contaminated environment in the Baía de Todos os Santos, with the objective of investigating the catabolic potential for aromatic hydrocarbon degradation. Functional annotation identified complete metabolic routes associated with the degradation of monoaromatics (styrene, xylene, and benzoate), in addition to the xenobiotic metabolism pathway mediated by cytochromes P450, indicating the presence of broad-spectrum oxidative enzymes. This catabolic capacity was validated experimentally by geochemical analysis (GC-MS) of the complete consortium, which proved high efficiency in the degradation of recalcitrant PAHs (Polycyclic Aromatic Hydrocarbons), including Acenaphthylene (98%) and Chrysene (70%). These results suggest that the consortium operates according to a cooperative functional model, in which different species share complementary steps of oxidation, transformation, and mineralization of aromatic hydrocarbons. Thus, the study provides genomic evidence, corroborated experimentally, of the consortium's biotechnological potential for application in the bioremediation of environments contaminated by petroleum and its derivatives.

1. Introduction

Bioremediation has established itself, since the 1980s, as a sustainable and effective alternative for the treatment of environments contaminated by organic and inorganic pollutants. This approach gained prominence after its application in the Exxon Valdez oil tanker disaster, which occurred in 1989, that evidenced the potential of microorganisms in the degradation of complex and persistent contaminants, such as polycyclic aromatic hydrocarbons (PAHs) derived from petroleum (Baniasadi; Mousavi, 2018; Young, 2023).

Since then, the growth in global demand for petroleum and its derivatives, which according to OPEC estimates may reach an average of 110 million barrels per day by 2045, allied with the increased risks of spills during its prospecting, transport, and refining, reinforces the urgent need for more ecological and efficient strategies to mitigate the environmental impacts of these accidents (“Global oil products demand outlook 2050 | Statista”, 2025). Such events represent serious threats to aquatic and coastal ecosystems, as PAHs are among the main responsible parties for the toxic, mutagenic, and carcinogenic effects associated with petroleum contamination (Gkorezis et al., 2016).

PAHs constitute a class of organic aromatic compounds formed by two or more condensed benzene rings, characterized by their hydrophobicity and low water solubility, which confers high environmental persistence upon them. The biological degradation of these compounds occurs predominantly under aerobic conditions, mediated by oxygenase and dehydrogenase enzymes that catalyze the initial steps

of activation and opening of the aromatic ring, converting them into simpler and less toxic intermediates (Amini; Giyahchi; Moghimi, 2024).

The efficiency of this process is frequently amplified by the action of microbial consortia, which bring together species with complementary metabolic pathways, capable of acting cooperatively in the oxidation and mineralization of PAHs of different sizes and structures (Mahjoubi et al., 2023; Parus et al., 2023). This metabolic synergy is essential for the complete degradation of these recalcitrant compounds, since few microorganisms in isolation possess all the genes and enzymes necessary for the catalysis of the multiple steps involved (Paniagua-Michel; Fathepure, 2018).

Microbial consortia isolated from naturally contaminated environments, such as mangroves, present particular relevance in this context. Such ecosystems, subject to variable salinity and the continuous presence of hydrocarbons, favor the selection of resistant and metabolically versatile microorganisms, capable of degrading PAHs under adverse conditions. Furthermore, many of these strains produce metabolites of biotechnological interest, such as biosurfactants, which reduce surface tension and facilitate access to the hydrophobic compounds (Costa et al., 2007; Parus et al., 2023).

Despite experimental advances, gaps still persist in the understanding of the molecular and genetic mechanisms that sustain PAH biodegradation. This limitation stems from the complexity of microbial interactions and the diversity of enzymes involved in the different steps of aromatic oxidation (Rawat; Rangarajan, 2019; Che; Men, 2019). In this sense, approaches based on functional genomics emerge as fundamental tools to elucidate metabolic routes, identify key genes and enzymes, and understand the specific role of each microorganism within the consortium.

In this way, the present study aimed to investigate the genomic and functional potential of a PAH-biodegrading bacterial consortium, isolated from a petroleum-contaminated mangrove. Through the complete sequencing of the genomes of the integrating strains and by comparative and functional analyses, it was sought to identify the main metabolic pathways related to PAH degradation and to understand the cooperative interactions that sustain the collective performance of the consortium.

2. Methodology

2.1 Biological Material

The bacteria used in this study are part of a mixed microbial consortium composed of 36 organisms previously isolated from contaminated sediments from the Baía de Todos os Santos (Bahia, Brazil), and is protected by a national patent (Lima et al., 2021; INPI BR 10 2021 002341 4). For the present investigation, 9 bacterial strains were selected based on taxonomic diversity and preliminary metabolic profile. The taxonomic identifications were performed from the analysis of the 16S rRNA region, associated with classic microbiology techniques, and the respective genera are listed in Table 1.

Table 1. Taxonomic identification of the bacterial strains selected for genomic analysis.

Sample Code	Access Number (NCBI)	Closest Relative Species	Similarity (%)
RB4	MW881197	Bacillus sp1	100
OH4	MW881204	Bacillus sp1	100
RC5	MW881198	Brevibacillus sp1	100
RA2	MW881196	Pseudomonas sp1	100
PD6	MW881201	Pseudomonas sp2	100
PD7	MW881202	Pseudomonas sp2	100
RC6	MW881199	Stenotrophomonas sp1	100
PD5	MW881200	Stenotrophomonas sp1	100
PD8	MW881203	Stenotrophomonas sp1	100

2.2 Genomic sequencing and data processing

2.2.1 Genome sequencing

The genomic sequencing was performed by a third-party service at the University of Göttingen (Germany), using the Illumina HiSeq 2500 platform, with generation of 2 × 150 bp paired-end reads and inserts of approximately 500 bp. The quality of the reads was verified with FastQC, considering Phred values $\ge$ 30. Next, pre-processing was performed with Fastp (Chen et al., 2018) for adapter removal and filtering of low-quality bases. The de novo assembly of the genomes was conducted in Unicycler, and the evaluation of quality metrics, such as N50, number of contigs and coverage, was done with QUAST (Gurevich et al., 2013).

2.2.2 Genomic annotation

The functional annotation of the bacterial genomes was conducted in an integrated manner using the RASTtk (Rapid Annotation using Subsystem Technology) pipelines, accessed via BV-BRC (Bacterial and Viral Bioinformatics Resource Center), and Prokka. The consolidated annotations were subsequently submitted to GhostKOALA for attribution of KO numbers and reconstruction of metabolic pathways in KEGG Mapper, with the results being compared and validated in PATRIC (Pathosystems Resource Integration Center).

Additionally, the data were enriched with information from complementary databases, UniProt, InterProScan, Gene Ontology (GO), and BRENDA, ensuring greater precision in the identification of genes

and enzymes related to the degradation of aromatic organic compounds..

2.2.3 Comparative analysis

Based on the annotated data, a functional analysis was performed, focused on the detection of genes and metabolic pathways involved in the biodegradation of aromatic hydrocarbons. The comparative analyses between the strains considered the presence or absence of structural and regulatory genes for mono- and dioxygenases, catechol- and benzoate-dioxygenases, and other central enzymes of the PAH degradation routes. The functional organization of the data was visualized by means of heatmaps and gene distribution tables generated in the BV-BRC, allowing to infer the individual and collective catabolic potential of the strains. Promoter regions of genes of interest were predicted with BPROM (Softberry), with the objective of estimating the gene expression potential under xenobiotic stress conditions.

To validate and correlate this genomic potential, the inferred catabolic profiles were compared with standardized experimental geochemical degradation data for the analyzed consortium (bacteria and fungi). These data, obtained by gas chromatography coupled to mass spectrometry (GC-MS), quantified the degradation rates at time points of 0, 16, and 30 days (T0, T1, and T2, respectively) of the 16 priority PAHs. The objective of the comparative analysis was, therefore, to connect the presence of functional genomic pathways with the observed performance in the removal of the respective aromatic substrates.

3. Results

3.1 Complete genome sequencing and genome assembly

The complete genomic sequencing was performed for the nine bacterial strains integrating the microbial consortium, generating high-quality data and adequate coverage for de novo assembly of the genomes, as described in Table 2. The quality control and assembly steps, conducted with the FastQC, Fastp, and Unicycler tools, were evaluated by means of QUAST, as detailed in the methodology.

Table 2. Quality parameters of the sequencing and assembly of the bacterial genomes.

Cepas	Gêneros	Contigs	GC % Content	N50	Genome size (bp)	CDS
RB4	Bacillus sp1	57	35.26	320,098	5,397,725	5,681
OH4	Bacillus sp1	34	35.07	1,245,830	5,269,420	5,35
RC5	Brevibacillus sp1	37	47.25	480,992	6,158,077	5,703
RA2	Pseudomonas sp1	71	56.44	490,574	6,600,291	6,511
PD6	Pseudomonas sp2	218	53.98	97,114	9,211,629	8,492
PD7	Pseudomonas sp2	31	43.67	1,039,881	4,098,655	4,106
RC6	Stenotrophomonas sp1	254	58.22	106,728	11,738,852	10,693
PD5	Stenotrophomonas sp1	166	58	212.574	13.441.238	12.005
PD8	Stenotrophomonas sp1	76	56.32	371,22	6,588,034	5,891

The assembled genomic sizes, the GC content rates, and the total number of CDS (Table 2) presented expressive variations among the different genera, reflecting the structural diversity of the consortium, but remaining within the expected values for their respective taxonomic classifications.

The number of contigs varied from 31 to 254, indicating different levels of assembly fragmentation. The strains from the Bacillus and Brevibacillus genera presented the lowest numbers of contigs, suggesting greater genomic continuity and assembly quality, reinforced by the high N50 values. The GC content values showed themselves to be consistent with those reported for close species, and the size of the genomes (4.09 to 13.44 Mbp) maintained proportionality with the number of coding regions (CDS), an expected pattern due to the variation in gene size between distinct genera.

3.2 Comparative analysis

The functional analysis of the annotated metabolic pathways in the genomes of the nine strains revealed a diversified and functionally rich profile, with highlight to routes associated with the degradation of aromatic compounds and xenobiotics derived from petroleum. Figure 1 presents a general overview of the distribution of genes and enzymes (ECs) involved in relevant metabolic pathways, evidencing the broad collective catabolic capacity of the consortium.

Among the annotated pathways, the degradation routes for benzoate, toluene, xylene, styrene, and PAHs, such as anthracene, fluorene, and pyrene, stood out, in addition to generalist pathways associated with xenobiotic metabolism by cytochrome P450. Complementary routes related to the degradation of persistent industrial compounds, such as 1,4-dichlorobenzene, trinitrotoluene, and caprolactam, were also identified.

This profile demonstrates that the consortium presents a set of specific and synergistic metabolic routes geared towards the transformation of complex aromatic compounds, including intermediates of PAHs, by means of initial oxidative mechanisms and of pathways converging to the citric acid cycle.

Furthermore, the contrast between the high total number of genes per pathway and the limited number of distinct ECs suggests the presence of functional gene redundancy, a characteristic trait of environmental microbial consortia, which confers metabolic resilience and capacity for adaptation to different substrates (Top et al., 2020).

3.2.1 Biodegradation pathways and xenobiotic metabolism

Figure 2. Heatmaps of the metabolic pathways related to biodegradation and xenobiotic metabolism in the microbial consortium.

The genomic analysis of the microbial consortium revealed a diversified metabolic profile with potential for the degradation of aromatic compounds derived from petroleum. Complete or partial routes were identified for benzoate, styrene, toluene, xylene, and substituted aromatic intermediates, with different levels of integrity and broad functional distribution among the strains.

Likewise, the styrene degradation pathway (figures 2 and 3), presented itself as robustly represented, with all the essential enzymes for the aerobic conversion of the compound into fumarate and acetoacetate, by the initial oxidation of the vinyl side chain, in addition to diverse conversion sub-routes. With highlight to phenylacetaldehyde dehydrogenase (EC 1.2.1.39), identified in Brevibacillus (RC5) and associated with regulation by oxidative stress factors, such as SoxS.

Figure 3. Map of the metabolic pathway of styrene degradation for the microbial consortium.

In the xylene degradation pathway (figures 3 and 5), great completeness was visualized, especially in the sub-routes of oxidation of benzylic alcohols to aldehydes with subsequent formation of benzoic/methylbenzoic acids (p-xylene, o-xylene, m-xylene). Because, despite the absence of the toluene methyl-monooxygenase (EC 1.14.15.26), responsible for catalyzing the first step in the degradation of toluenes and xylenes, the result suggests an integral degradation of these compounds occurs at the consortium level, by functional cooperation between lineages.

The xylene pathway also stands out, for its importance in participating in the degradation of toluene, which has the option of an aerobic sub-route via oxidation of the side chain, followed by ring opening via catechol ortho-cleavage, falling into the xylene degradation pathway, specifically in the degradation branch via cis,cis-muconate (3-methyl-cis,cis-muconate), which is processed by muconate-lactonization and subsequent hydrolysis.

Figure 4. Map of the metabolic pathway of xylene degradation for the microbial consortium.

In turn, the degradation of benzoate (figures 4 and 5), presented itself as widely distributed among the different strains and with 3 main specific modules represented: The catechol ortho-cleavage sub-pathway, a hydroxylation pathway for benzoate with the presence of genes like 1,2-dioxygenase and catechol dioxygenase resulting in acetyl-CoA/succinyl-CoA and entering the citric acid cycle (TCA), a

classic aerobic pathway for mineralization of aromatics; The catechol meta-cleavage, an adapted variation for hydroxylated derivatives, and which can generate pyruvate (glycolysis) or enter the TCA; And the benzyl-CoA sub-route, or benzoate degradation route, which passes through the aromatic β-oxidative cycle, destined for the citric acid cycle, with this module being more common in anaerobic or microaerobic conditions, processing aromatics via CoA activation.

Furthermore, the benzoate degradation pathway revealed itself as the central point in the integration of diverse metabolic routes, such as: aminobenzoate, with a complete sub-pathway to its metabolization into pyruvate or TCA; the connection with the fluorobenzoate pathway, receiving intermediate compounds (maleylacetate and catechol) from the fluorocatechol routes, with highlight of representation for strains PD5, PD6, and RC6; Toluene, which also presents the benzoate degradation pathway, an oxidative route via the benzylic side chain, resulting in benzoate; And chlorinated derivatives such as 1,4-dichlorobenzene, 2,4-dichlorobenzoate, and tetrachloroethene, which are partially represented, with highlight to the RA2 strain (Pseudomonas), responsible for activating halogenated substrates via haloalkane dehalogenase.

Figure 5. Map of the metabolic pathway of benzoate degradation for the microbial consortium.

Regarding the polycyclic aromatic hydrocarbons, such as anthracene, fluorene, and pyrene, the regular degradation pathways were not detected in the analyzed genomic sequences. However, the presence of multiple genes belonging to the xenobiotic metabolism pathway by cytochrome P450 (KEGG map00980) was identified. These genes, which are found to be widely distributed among the strains, indicate a potential for initial activation of xenobiotics by oxidation, which could generate intermediates assimilable by the specific metabolic routes already annotated, such as that of catechol.

In parallel with the genomic analysis, the experimental performance of the complete consortium in the degradation of 10 priority PAHs that the consortium demonstrated the capacity to degrade was quantified (Figure 6).

Figure 6. Degradation rates of priority PAHs by the complete microbial consortium, analyzed by GC-MS.

The geochemical results (Figure 6) demonstrated a high removal rate for the majority of the PAHs analyzed. The total degradation (T0-T2) reached high values for 2 and 3-ring compounds, such as Acenaphthylene (98%), Acenaphthene (97%), Phenanthrene (85%), and Anthracene (87%). Additionally, the consortium showed itself to be competent in the removal of 4-ring PAHs, with degradation rates of 80% for Pyrene and 70% for Chrysene.

4. Discussion

4.1 Evaluation of genome assembly and structural diversity

The analysis of the genome assembly data from the nine bacterial strains of the consortium revealed parameters consistent with similar studies on genomic characterization of environmental lineages (Tully et al., 2018; Parks et al., 2020). The observed variation in genomic size (4 to 13 Mbp) is in accordance with what is expected for free-living bacteria with high metabolic plasticity. The strains belonging to the Stenotrophomonas and Pseudomonas genera presented relatively larger genomes and CDS numbers, which is in conformity with the elevated metabolic potential associated with these genera in the literature, especially in processes of aromatic compound degradation and bioremediation (Singh; Saini; Kahlon, 2016; Jiménez et al., 2004).

The assembly parameters, such as N50 and number of contigs, presented wide variation, but remained within the expected standards for sequencing performed with short-reads. The use of tools like Unicycler and QUAST contributed to the reliability of the obtained assemblies. However, the adoption of hybrid approaches with long-reads (such as Oxford Nanopore) can be considered in future studies to reduce the observed fragmentation, especially in larger-sized genomes, where the presence of mobile genetic elements and repetitive regions tends to hinder genomic closure (Orellana et al., 2023).

The variation in GC content among the strains reflects the diversified phylogenetic composition of the consortium, which is advantageous in contaminated environmental contexts. This heterogeneity allows for the functional distribution of catabolic pathways among organisms with different physiological strategies and chemical stress response mechanisms. In this way, the genomic assemblies presented sufficient robustness for functional annotation analyses, metabolic pathway reconstruction, and identification of key genes associated with the degradation of aromatic hydrocarbons and xenobiotics (Pandolfo et al., 2024; Rojas-Vargas et al., 2024).

4.2 Comparative genomic analysis

The functional genomic characterization of the consortium revealed a highly diversified and redundant metabolic structure, with complementary routes geared mainly towards the degradation of aromatic compounds and xenobiotics derived from petroleum. This pattern is typical of microbial consortia adapted to contaminated environments, in which different lineages share catabolic steps and cooperate metabolically to promote the degradation of complex mixtures (Mohapatra et al., 2022; Pérez-Pantoja et al., 2016).

Among the most relevant results, the widely distributed presence of genes associated with the xenobiotic metabolism pathway mediated by cytochromes P450 stood out. This route represents a classic phase I mechanism of bacterial detoxification, responsible for the initial oxidation of lipophilic and recalcitrant compounds, such as naphthalene, toluene, styrene, and benzo[a]pyrene, without the need for complete mineralization pathways (Guengerich, 2008). The oxidation catalyzed by CYP-type monooxygenases, followed by the action of epoxide hydrolases and associated reductases, generates more polar intermediates amenable to assimilation by central pathways, constituting a microbial survival and adaptation strategy when facing toxic compounds (Hossain et al., 2024).

However, the presence of these bacterial P450 genes does not fully explain the observed performance in PAH degradation. As demonstrated in the geochemical results (Fig 7), the complete consortium exhibited high efficiency in the removal of 2- to 4-ring PAHs, such as anthracene and chrysene. Nonetheless, the genomic analysis of the bacterial strains did not identify the canonical ring-hydroxylating dioxygenase pathways, which are the main bacterial mechanisms for the initial attack on these PAHs. (Debajyoti ghosal et al., 2016) This finding suggests a metabolic gap: the consortium's bacteria possess the 'lower pathways' to mineralize the intermediates, but not those to initiate the attack on the polycyclic PAHs.

This apparent contradiction is resolved when considering the complete nature of the consortium, which includes not only the analyzed bacteria, but also fungi. The scientific literature describes fungi as potent PAH degraders, precisely because they do not depend on specific assimilation pathways (Ghosal et al., 2016). Instead, fungi utilize extracellular and non-specific enzymatic systems, such as laccases and peroxidases, to perform the initial oxidative attack. A fungi-bacteria synergy model is therefore proposed: the consortium's fungi perform the initial and non-specific oxidation of PAHs by cleaving the rings, and the bacteria metabolize the soluble, low-molecular-weight intermediates generated by this breakage. (Katarzyna Wróblewska; Jeong, 2021)

Besides the oxidative routes, pathways with great gene completeness or visibly described for the degradation of compounds like styrene, xylene, toluene, benzoate, and their derivatives were identified, demonstrating that the consortium also possesses genomic competence for aerobic mineralization by specific routes.

Among them, the degradation of styrene is substantiated by the presence of genes that allow its conversion into acetoacetate and fumarate, intermediates of the citric acid cycle. Standing out is the Brevibacillus strain (RC5), which, despite not belonging to lineages traditionally associated with hydrocarbon degradation, presented relevant unique genes, such as phenylacetaldehyde dehydrogenase, with active promoters regulated by factors like SoxS, typical of responses to oxidative, and consequently environmental, stress. (Vieto et al., 2021; Brzeszcz; Kaszycki, 2018).

The degradation of toluene revealed two possible sub-routes, functionally integrated with the xylene and benzoate pathways, forming a cooperative catabolic system between strains. Toluene can be oxidized via 3-methylcatechol or converted to benzoate, which in turn is transformed into TCA cycle intermediates. This arrangement suggests that the complete mineralization of these compounds depends on the metabolic interaction between different members of the consortium. A similar pattern was observed for naphthalene derivatives, such as 2-methylnaphthalene, in which incomplete routes were compensated for by convergent pathways or by the exchange of metabolic intermediates between species, a central phenomenon in the functional synergy of microbial consortia (Moran et al., 2023).

The xylene pathway, although presenting the absence of the enzyme toluene methyl-monooxygenase (EC 1.14.15.26), showed itself to be practically complete, indicating that the initial oxidation may occur via non-specific enzymes, such as the monooxygenases of the P450 system. This type of catalytic

flexibility is a common trait in environmental bacteria and reinforces the adaptive potential of the consortium against compounds of variable structure.

The benzoate pathway was one of the most stable and widely distributed among the strains, consolidating itself as the convergent nucleus of aromatic metabolism. Enzymes such as benzoate 1,2-dioxygenase and catechol dioxygenases were identified, essential for the aerobic mineralization of these compounds via ortho- and meta-cleavage sub-routes, resulting in the production of acetyl-CoA, succinyl-CoA, and pyruvate. Furthermore, the presence of the benzyl-CoA route was observed, typically associated with microaerobic conditions, which reinforces the physiological versatility of the consortium (Harwood; Parales, 1996; Alexander, 1999).

Together, the data suggest that the studied bacterial consortium adopts a mixed functional model, in which different species cooperate in complementary steps of oxidative activation, intermediate transformation, and final mineralization of aromatic compounds. The xenobiotic metabolism pathways mediated by cytochromes P450 assume a central role in the initial oxidation of PAHs and derivatives, while the catechol and benzoate routes sustain the subsequent aerobic mineralization.

This metabolic integration between generalist and specific pathways, associated with gene redundancy and interspecies complementarity, characterizes the consortium as a system adapted to environments contaminated with petroleum and its derivatives, with high potential for application in the bioremediation of complex aromatic hydrocarbons (Pandolfo et al., 2024; Guntupalli et al., 2016; Malik; Ahmed, 2012).

5. Conclusion

The functional genomic analysis of the nine bacterial strains revealed a robust and diversified metabolic arsenal for the degradation of aromatic hydrocarbons. The bacterial consortium demonstrated possessing the complete central pathways for the mineralization of monoaromatic compounds, such as styrene, xylene, and, crucially, benzoate, which acts as the main convergent intermediate. Furthermore, the presence of genes for cytochromes P450 indicates a versatile capacity for the initial oxidation of xenobiotic compounds.

This genomic potential was validated experimentally by the geochemical analysis (GC-MS) of the complete consortium, which confirmed a high removal rate of recalcitrant PAHs of 2 to 4 rings. Together, the data demonstrate that the consortium operates by a functional cooperation model, where different bacterial strains contribute with complementary steps for the catabolism of complex compounds, consolidating its biotechnological potential for the bioremediation of contaminated environments.

Declarations

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Figures

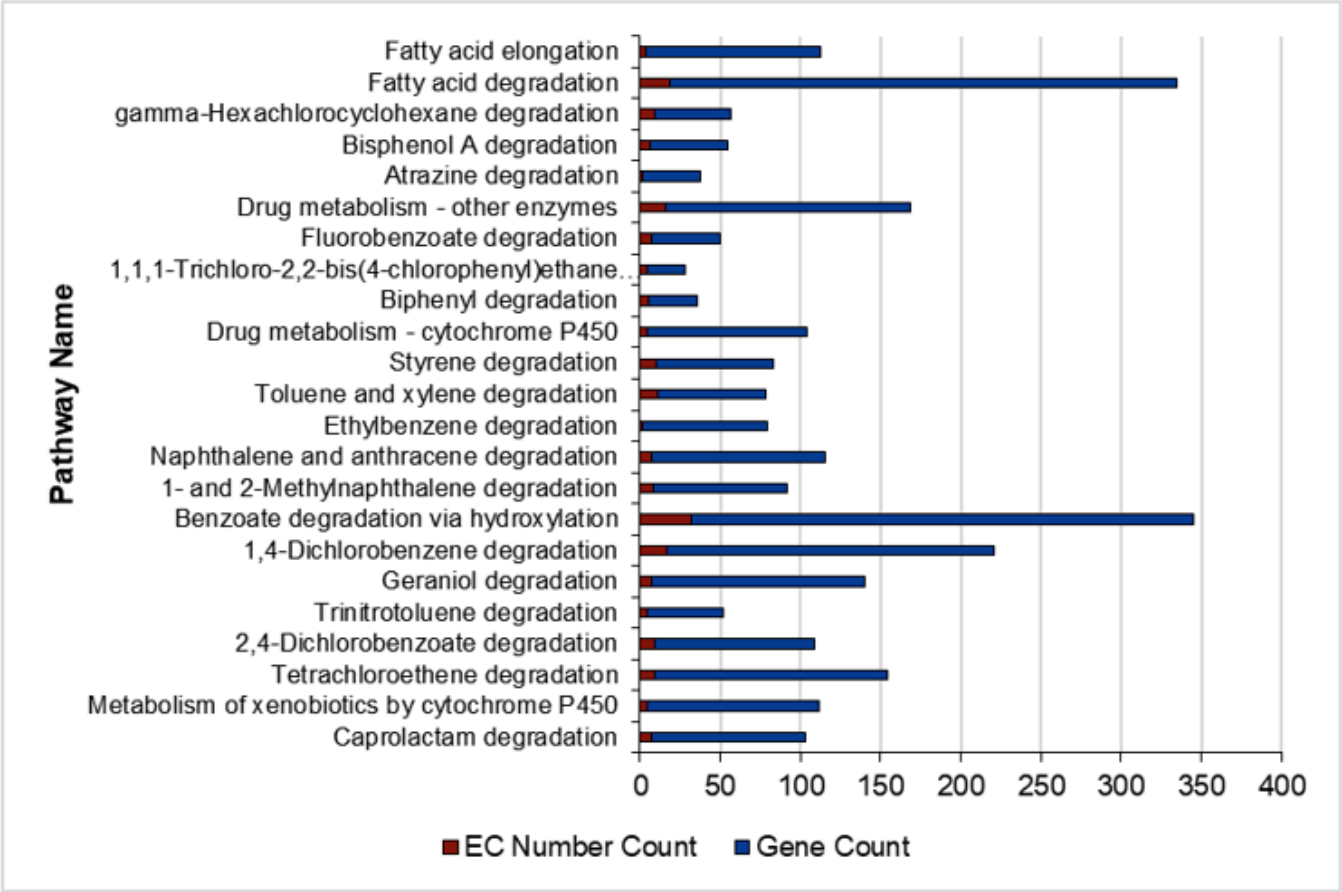


Figure 1

Quantity of genes and enzymes (ECs) annotated in metabolic pathways related to the degradation of petroleum derivatives.

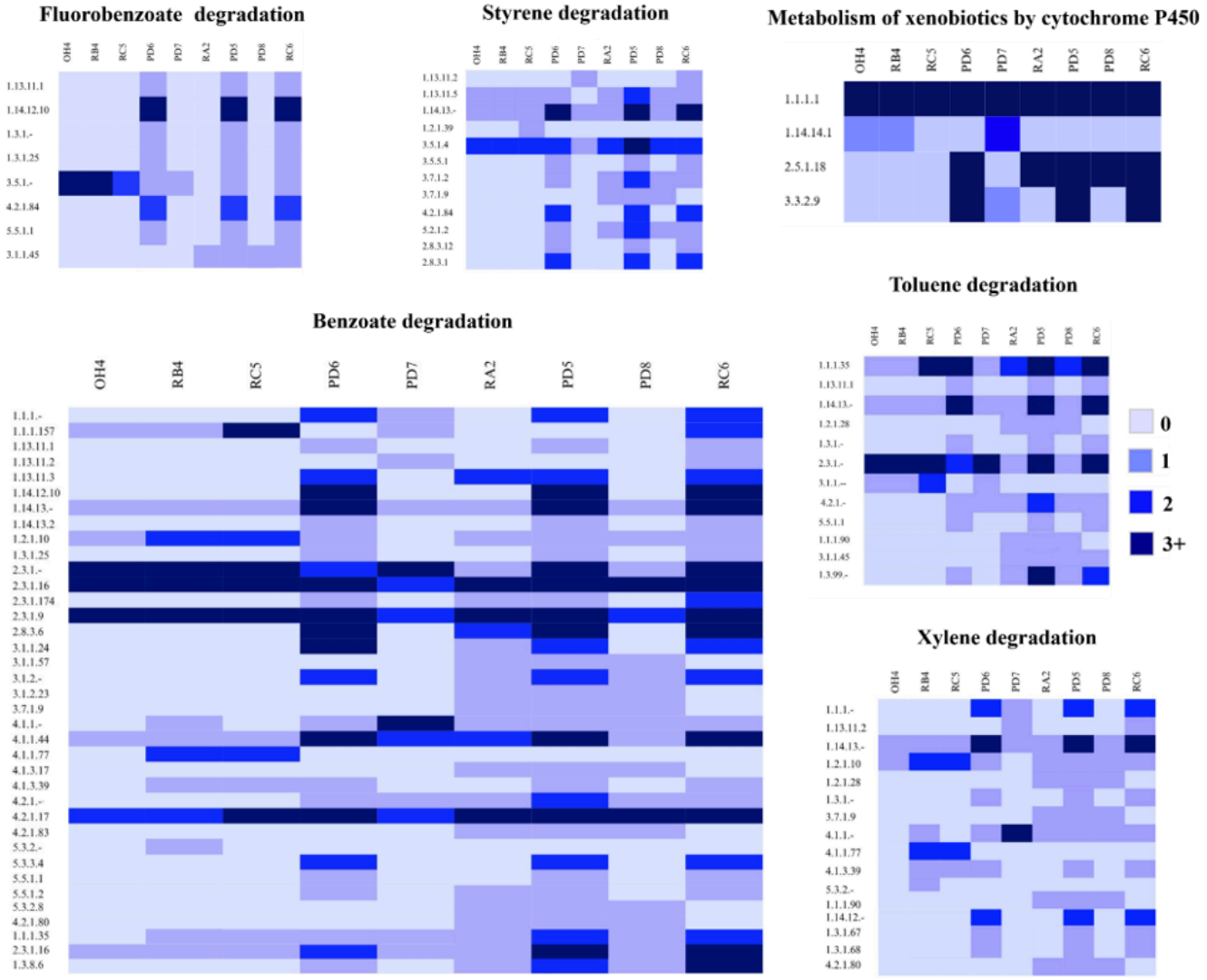


Figure 2

Heatmaps of the metabolic pathways related to biodegradation and xenobiotic metabolism in the microbial consortium.

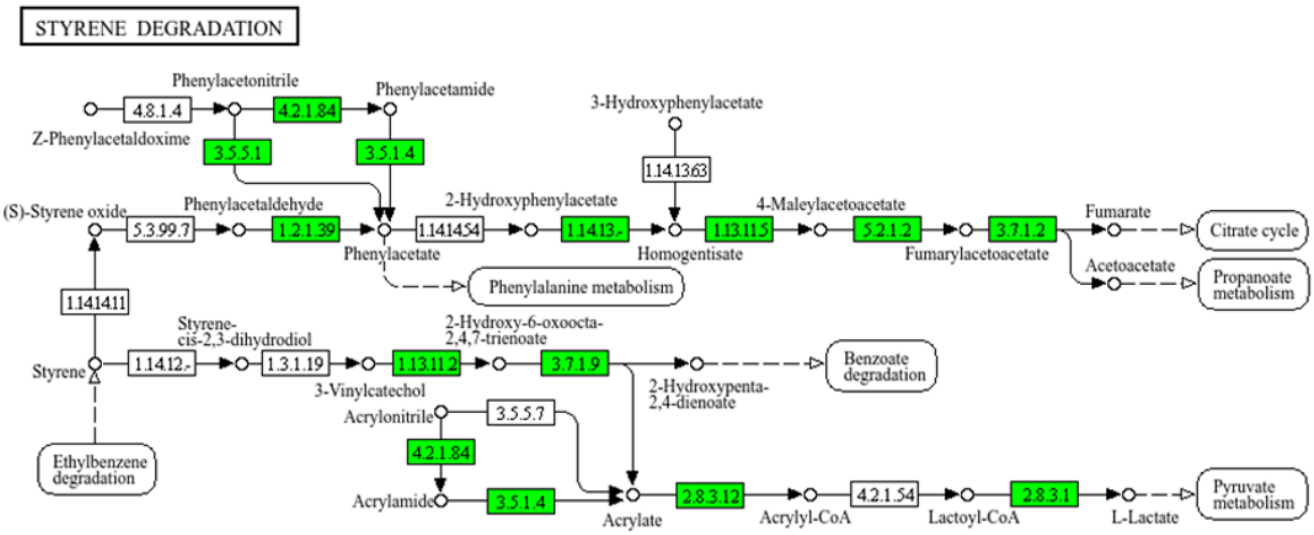


Figure 3

Map of the metabolic pathway of styrene degradation for the microbial consortium.

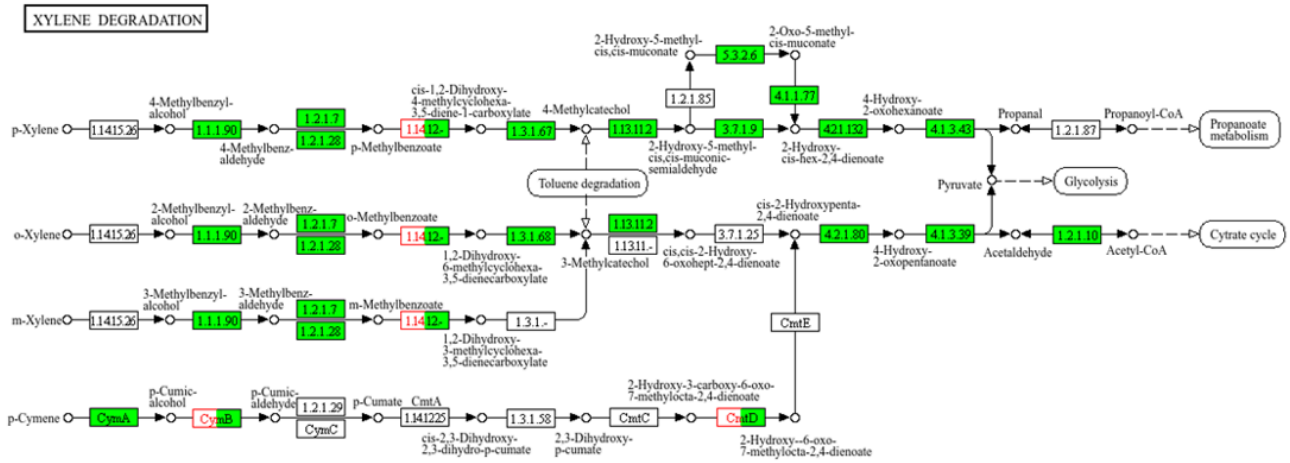


Figure 4

Map of the metabolic pathway of xylene degradation for the microbial consortium.

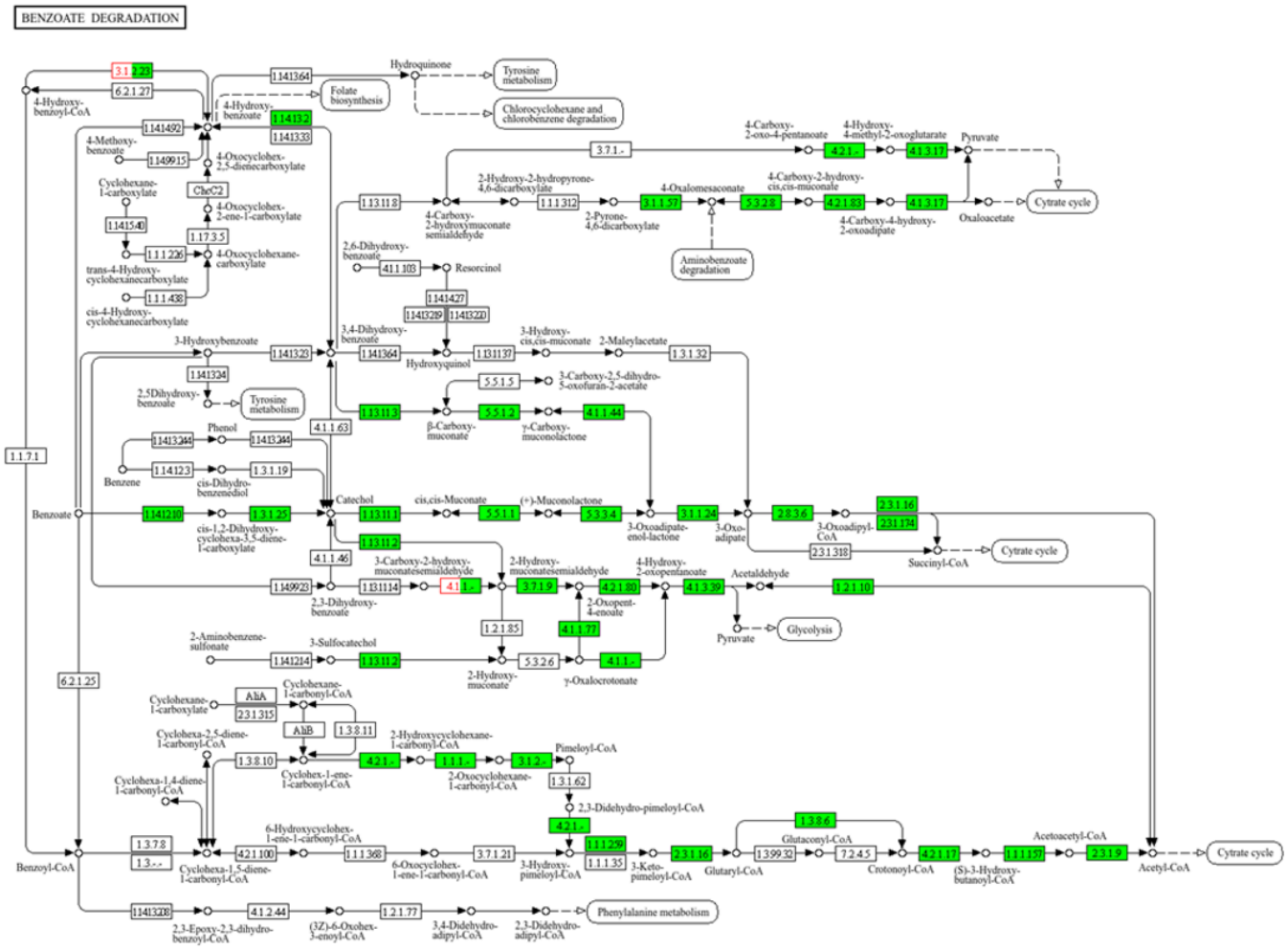


Figure 5

Map of the metabolic pathway of benzoate degradation for the microbial consortium.

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Figure 6

Degradation rates of priority PAHs by the complete microbial consortium, analyzed by GC-MS.

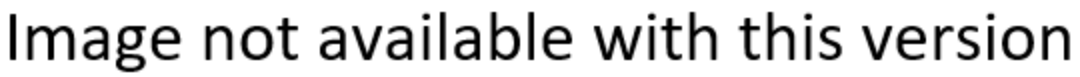


Figure 7

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