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REVIEW

Unlocking microbial potential: advances in omics and bioinformatics for aromatic hydrocarbon degradation

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Abstract

Aromatic hydrocarbons (AHs) are persistent environmental pollutants with high toxicity. Bacterial degradation of AHs provides a sustainable and cost-effective approach for the remediation of sites contaminated with both mono- and polycyclic aromatic hydrocarbons. Aerobic degradation of AHs typically involves oxygenases-mediated hydroxylation followed by aromatic ring cleavage. In contrast, anaerobic degradation relies on diverse activation mechanisms that ultimately converge on the central intermediate benzoyl-CoA. Over the past decades, research on bacterial degradation of AHs has grown steadily, supported by advances in omics and bioinformatics. In this review, we summarize the current knowledge on the pathways, enzymes, and microbial diversity involved in AH degradation, highlighting how omics and bioinformatic approaches are advancing our understanding of this process. However, to improve our knowledge of microbial AHs catabolism, it is crucial to prioritize the characterization of novel enzymes and pathways, especially those mediating anaerobic and hybrid degradation strategies. Addressing this gap requires the development of specialized resources that incorporate a broader taxonomic diversity and an expanded inventory of anaerobic genes and enzymes supported by experimental evidence. Equally important is the integration of multi-omics technologies, artificial intelligence, and ecological modeling into unified analytical pipelines. These efforts will be key to fully unlocking microbial metabolic potential and guiding more effective bioremediation and monitoring strategies for AHs.

Keywords Monocyclic aromatic hydrocarbons · Polycyclic aromatic hydrocarbons · Microbial degradation · Aerobic and anaerobic · Bioinformatic tools · Genomics and metagenomics

Introduction

Aromatic hydrocarbons (AHs) are organic compounds with at least one benzene ring as a central structural unit. They are broadly divided into monocyclic aromatic hydrocarbons (MAHs) and polycyclic aromatic hydrocarbons (PAHs). MAHs contain a single benzene ring, making them the simplest form of aromatic compounds (Shrivastava and Phale 2013; Ladino-Orjuela et al. 2016). This category includes benzene, toluene, ethylbenzene, and xylene, collectively referred to as BTEX (Shrivastava and Phale 2013; Phale et al. 2020). In contrast, PAHs consist of two or more fused aromatic rings without any heteroatoms. PAHs containing up to three rings are classified as low molecular weight (LMW) PAHs, while those with four or more rings are termed high molecular weight (HMW) PAHs. Over 100 PAHs and their derivatives have been identified, including LMW compounds such as naphthalene, fluorene, anthracene, and phenanthrene, and HMW representatives such as fluoranthene, pyrene, benzo[a]anthracene, and chrysene (Seo et al. 2009; Phale et al. 2020).

AHs are of significant concern due to their toxicity and ubiquity in all environments. Many BTEX and PAHs, along with their metabolized forms, are widely recognized as potent carcinogenic, mutagenic, and teratogenic agents. The carcinogenic properties of AHs are primarily related to their

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ability to form DNA adducts, which contribute to key gene mutations (Martins et al. 2013). In addition, acute exposure to AHs has been associated with oxidative stress, reproductive disorders, immunosuppression, and endocrine disruption, further complicating their adverse health effects (Cao et al. 2022).

AHs are recalcitrant compounds due to the high thermodynamic stability of the benzene ring. Nonetheless, certain bacteria have evolved specialized metabolic pathways that enable them to utilize AHs as sole sources of carbon and energy (Shrivastava and Phale 2013). Previous studies have summarized core biochemical, genetic, and genomic aspects of bacterial catabolism of AHs (Carmona et al. 2009; Fuchs et al. 2011; Phale et al. 2020; Pandolfo et al. 2023). Since then, research in this field has benefited from rapid advances in sequencing technologies, the expansion of databases, and the development of sophisticated bioinformatic tools. In this review, we summarize the current knowledge on the mechanisms of AH biodegradation and emphasize the role of bioinformatics and omics approaches in revealing novel pathways, enzymes, and microbial diversity, while also highlighting areas that require further research.

Microbial degradation of aromatic hydrocarbons

Bacterial catabolism of AHs, under both anaerobic and aerobic conditions, is organized into an upper (or *peripheral*) and lower (or *ring-cleavage*) pathway. In the upper pathway, diverse aromatic compounds are funneled into central intermediates such as catechol, protocatechuate, or benzoyl-CoA. In the lower pathway, these intermediates are dearomatized and cleaved, resulting in metabolites that can be used by bacteria in the production of biomass.

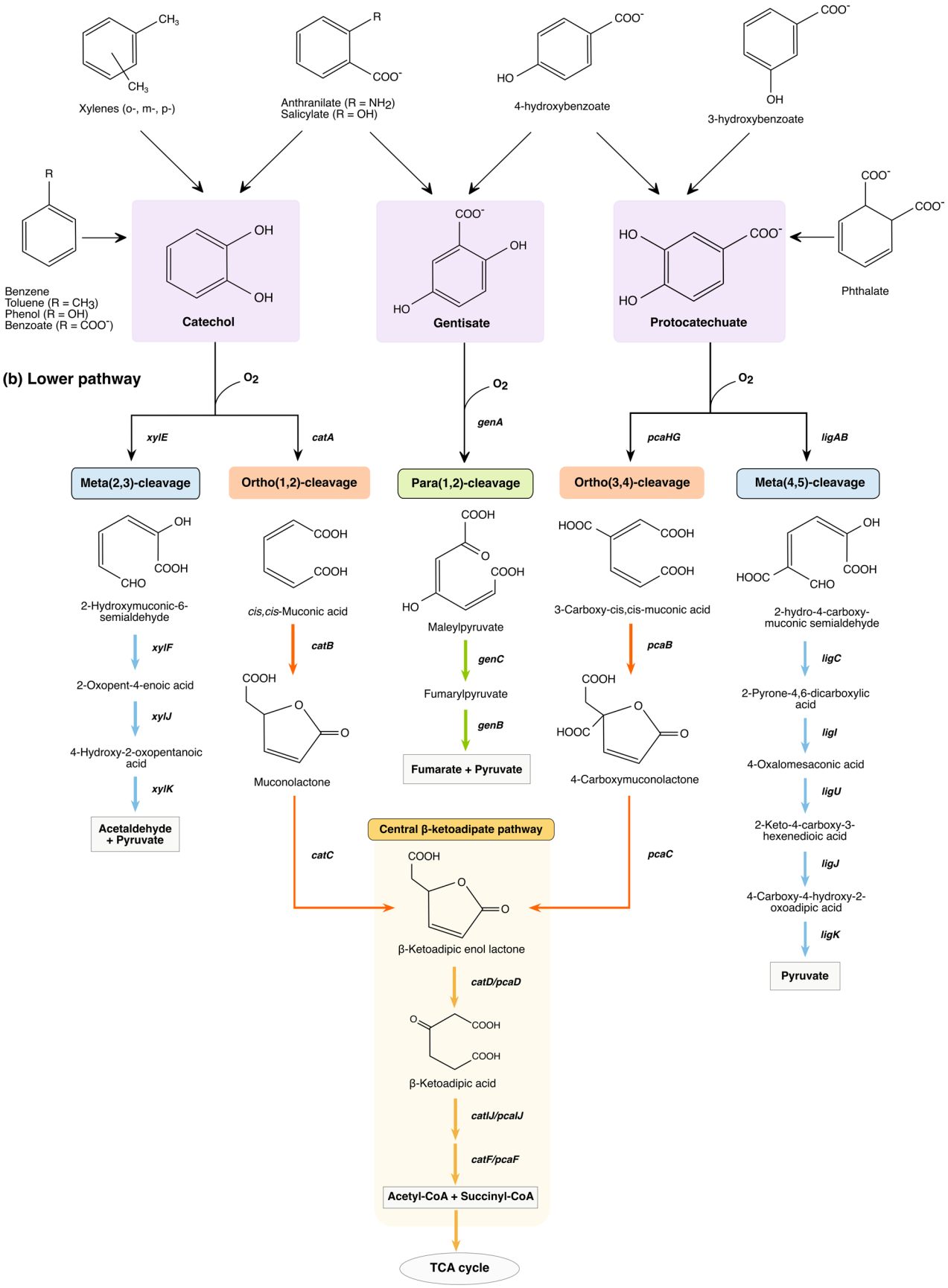
Aerobic catabolic pathways for the degradation of MAHs

A wide range of bacteria catalyze the biodegradation of AHs under oxic conditions, primarily including Gram-negative genera such as *Pseudomonas*, *Sphingomonas*, *Acinetobacter*, and *Ralstonia*, as well as Gram-positive genera like *Rhodococcus*, *Nocardioides*, and *Mycobacterium* (Phale et al. 2020). This process is mediated by oxygenases (monooxygenases and dioxygenases), which catalyze the incorporation of hydroxyl groups (-OH) into the aromatic ring using molecular oxygen (O_2) as a co-substrate. Monooxygenases incorporate one oxygen atom into the substrate while reducing the second to water. In contrast, dioxygenases insert both oxygen atoms into the aromatic ring, resulting in dihydroxylation or ring cleavage (Ladino-Orjuela et al. 2016).

Fig. 1 Aerobic catabolism of monoaromatic hydrocarbons. The process follows two main stages: in the upper (peripheral) pathways **(a)**, monoaromatic compounds are activated by monooxygenases or ring-hydroxylating dioxygenases to yield central hydroxylated intermediates (purple boxes). These compounds then enter the lower (central) pathways **(b)**, where ring-cleaving dioxygenases catalyze ortho-, meta-, or para-cleavage. The resulting ring-fission products (light-gray boxes), such as succinate, pyruvate, or acetyl-CoA precursors, are metabolized through the TCA cycle. The ortho-cleavage of catechol and protocatechuate converges into the common β-ketoadipic acid intermediate (β-ketoadipate central pathway). The cat cluster encodes enzymes for catechol ortho(1,2)-cleavage, including catechol 1,2-dioxygenase (*catA*), muconate cycloisomerase (*catB*), and muconolactone isomerase (*catC*). The *pca* cluster of the Protocatechuate ortho(3,4)-cleavage encodes a protocatechuate 3,4-dioxygenase (*pcaHG*), β-carboxy-cis, cis-muconate cycloisomerase (*pcaB*), and a γ-carboxy-muconolactone decarboxylase (*pcaC*). Downstream steps of β-ketoadipate pathway involve β-ketoadipate enol-lactonase (*catD/pcaD*), β-ketoadipate succinyl-CoA transferase (*catIJ/pcaIJ*) and β-ketoadipyl-CoA thiolase (*catF/pcaF*). In contrast, the *xyl* cluster directs the meta(2,3)-cleavage of Catechol through catechol 2,3-dioxygenase (*xylE*), 2-hydroxymuconic semialdehyde hydrolase (*xylF*), 2-hydroxymuconic semialdehyde dehydrogenase (*xylG*), 2-oxopent-4-enoate hydratase (*xylJ*), and a 4-hydroxy-2-oxalovalerate aldolase (*xylK*). Genes involved in the Protocatechuate meta(4,5)-cleavage encode a protocatechuate 4,5-dioxygenase (*ligAB*), 4-carboxy-2-hydroxymuconate-6-semialdehyde dehydrogenase (*ligC*), 2-pyrone-4,6-dicarboxylate hydrolase (*ligI*), 4-oxalomesaconate delta isomerase (*ligJ*), and 4-hydroxy-4-methyl-2-oxoglutarate aldolase (*ligK*). The cluster responsible for the Gentisate para(1,2)-cleavage pathway comprises genes encoding gentisate 1,2-dioxygenase (genA), maleylpyruvate isomerase (*genC*), and fumarylpyruvate hydrolase (*genB*)

The upper pathway begins with the activation of aromatic substrate, typically catalyzed by multicomponent ring-hydroxylating dioxygenases (RHDs) that produce cis-dihydrodiol derivatives (Fig. 1). These are subsequently oxidized to central intermediates such as hydroquinone, resorcinol, and catechol, the latter being the most common. Some pathways proceed via non-catecholic intermediates such as protocatechuate, gentisate, salicylate, and homoprotocatechuate (Ladino-Orjuela et al. 2016; Ghosal et al. 2016; Durante-Rodríguez et al. 2018). These hydrolated intermediates are then subjected to an ortho- or meta-cleavage via ring-cleaving dioxygenases (RCDs) in the lower pathway. Ortho-cleavage occurs between two hydroxyl groups and is catalyzed by intradiol dioxygenases. In contrast, meta-cleavage targets the adjacent C–C bond and is mediated by extradiol dioxygenases (Vaillancourt et al. 2006; Phale et al. 2020).

While meta-cleavage pathways exhibit considerable diversity, ortho-cleavage is primarily restricted to the central β-ketoadipate pathway (β-KAP). This pathway features separate branches for the catabolism of catechol and protocatechuate, both converging at β-ketoadipate enol-lactone


(a) Upper pathway
Xylenes (o-, m-, p-)
Anthranilate (R = NH2)
Salicylate (R = OH)
4-hydroxybenzoate
3-hydroxybenzoate
Benzene
Toluene (R = CH3)
Phenol (R = OH)
Benzoate (R = COO-)
Phthalate
Catechol
Gentisate
Protocatechuate
(b) Lower pathway
O2
xylE
catA
genA
pcaHG
ligAB
Meta(2,3)-cleavage
Ortho(1,2)-cleavage
Para(1,2)-cleavage
Ortho(3,4)-cleavage
Meta(4,5)-cleavage
2-Hydroxymuconic-6-semialdehyde
xylF
2-Oxopent-4-enoic acid
xylJ
4-Hydroxy-2-oxopentanoic acid
xylK
Acetaldehyde + Pyruvate
cis,cis-Muconic acid
catB
Muconolactone
Maleylpyruvate
genC
Fumarylpyruvate
genB
Fumarate + Pyruvate
3-Carboxy-cis,cis-muconic acid
pcaB
4-Carboxymuconolactone
2-hydro-4-carboxy-muconic semialdehyde
ligC
2-Pyrone-4,6-dicarboxylic acid
ligI
4-Oxalomesaconic acid
ligU
2-Keto-4-carboxy-3-hexenedioic acid
ligJ
4-Carboxy-4-hydroxy-2-oxoadipic acid
ligK
Pyruvate
catC
pcaC
Central β-ketoadipate pathway
β-Ketoadipic enol lactone
catD/pcaD
β-Ketoadipic acid
catIJ/pcaIJ
catF/pcaF
Acetyl-CoA + Succinyl-CoA
TCA cycle

(Wells and Ragauskas 2012). β-KAP is mostly chromosomally encoded (e.g., *pca* and *cat* genes in *P. putida*), but some exceptions exist, such as the plasmids pWW174 from *Acinetobacter calcoaceticus* and pSymB from *Sinorhizobium melilotii* (Shrivastava and Phale 2013). The pathway is broadly conserved across bacteria, yet its regulation and gene organization show notable diversification (Wells and Ragauskas 2012). For example, gene order within metabolic operons varies across bacterial species, except in cases where genes may coevolve due to their products being subunits of a single enzyme and are co-expressed. An example of this is the consecutive genes *pcaHG* and *pcaIJ*, which encode subunits of protocatechuate 3,4-dioxygenase and 3-oxoadipate CoA-transferase, respectively (Buchan et al. 2004) (Fig. 1).

Recent research has increasingly focused on RCDs, due to their critical role in determining pathway specificity during AH degradation. Notably, although the differences between intradiol and extradiol RCDs initially seemed minor, subsequent studies revealed that these enzymes belong to two distinct and evolutionary unrelated protein classes (Vaillancourt et al. 2006). Thus far, all characterized intradiol dioxygenases belong to the same superfamily. In contrast, sequence and structural analyses have identified three families of unrelated extradiol dioxygenases (Edos): Type-I (e.g., catechol 2,3-dioxygenases) within the vicinal oxygen-chelate (VOC) superfamily, Type-II in the LigB superfamily (e.g., protocatechuate 4,5-dioxygenases), and Type-III enzymes belonging to the cupin superfamily (e.g., gentisate dioxygenases) (Vaillancourt et al. 2006). Additionally, a benzoquinol 1,2-dioxygenase identified in *Pseudomonas fluorescens* ACB exhibits no significant sequence homology to known dioxygenases and has been proposed as the prototype of a novel Type-IV Edo (Pérez-Pantoja et al. 2010).

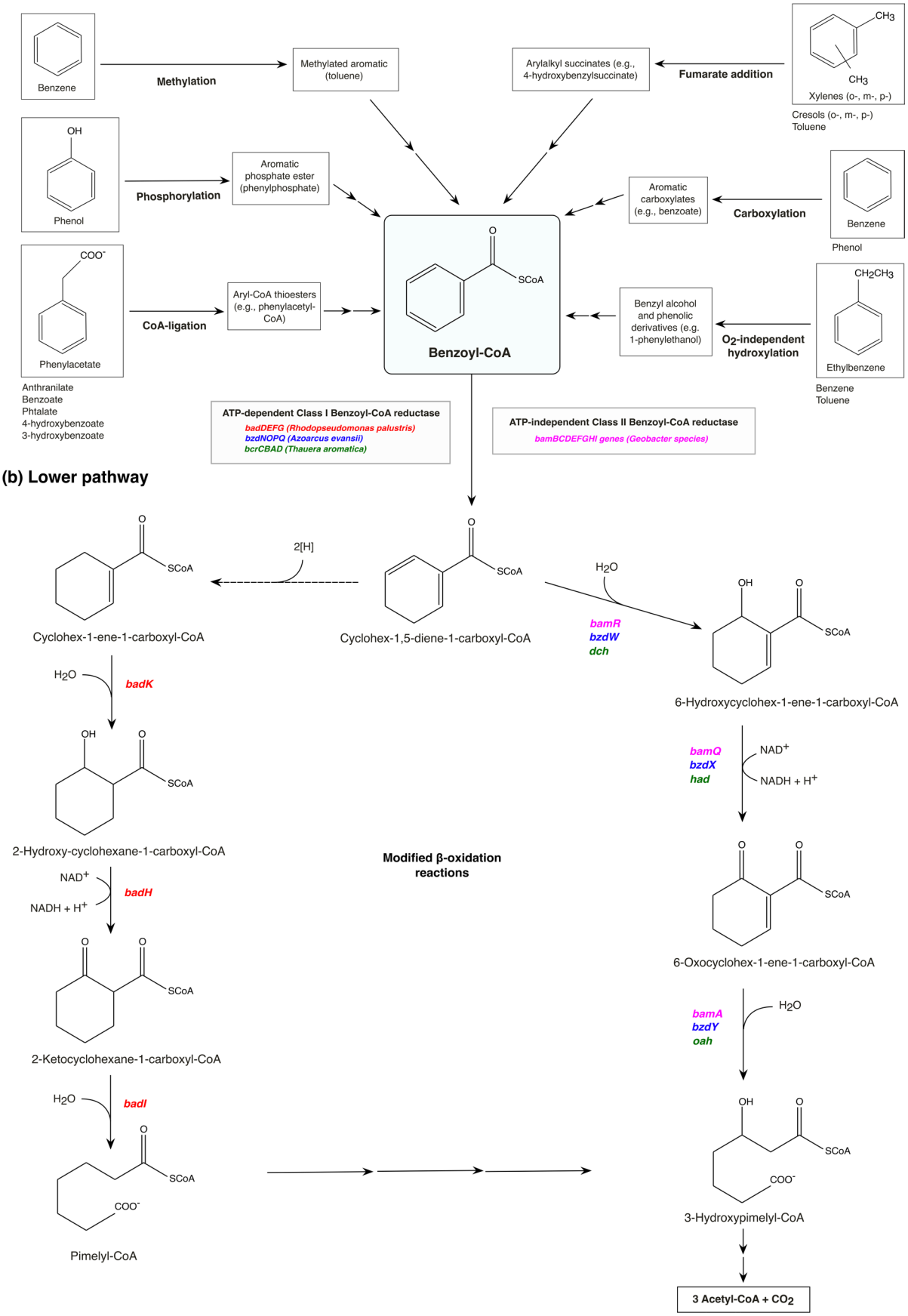
Type-III Edos are increasingly recognized for their role in the para-cleavage pathway, the third aerobic pathway for aromatic ring cleavage (Fig. 1). This pathway targets aromatic compounds with hydroxyl groups in the para-orientation (e.g., gentisates and hydroquinones) or those with a hydroxyl group adjacent to a carboxylate or amino group (e.g., salicylate, 2-aminophenol, and 1-hydroxy-2-naphthoate) (Ferraroni et al. 2013). An atypical type-III Edo is the gentisate 1,2-dioxygenase from *Pseudaminobacter salicylatoxidans* BN12. This enzyme shows broad substrate specificity, oxidizing gentisate and other monohydroxylated substrates (e.g., 1-hydroxy-2-naphthoate, substituted salicylates). These unique features have established type-III Edos as valuable models for exploring structure-function relationships within this family (Ferraroni et al. 2013; Subbotina et al. 2021).

Fig. 2 Anaerobic catabolism of monoaromatic hydrocarbons through the Benzoyl-CoA pathway. In the upper pathway **(a)**, diverse monoaromatic substrates are funneled into the common intermediate benzoyl-CoA (light-blue box) through distinct activation strategies including ATP-dependent CoA-ligation, phosphorylation, methylation, fumarate addition, carboxylation, and O_2-independent hydroxylation. Boxes illustrate representative aromatic compounds activated by each mechanism; additional substrates listed below each box are likewise activated by the same mechanism but display distinct chemical structures. In the lower pathway **(b)**, aromatic ring reduction of benzoyl-CoA is carried out by either an ATP-dependent Class I benzoyl-CoA reductase encoded by clusters such as *badDEFG* in *R. palustris*, *bzdNOPQ* in *A. evansii*, or *bcrCBAD* in *T. aromatica*; or by an ATP-independent Class II reductase encoded by the *bamBCDEFGHI* gene cluster (*Geobacter* species). Next in the pathway, the reduced intermediate can follow two slightly different β-oxidation-like pathways leading to the formation of pimelyl-CoA in *R. palustris* and 3-hydroxypimelyl-CoA in *Thauera*, *Azoarcus* and *Geobacter* species. Genes involved in each step are color-coded according to the corresponding model organism: red (*R. palustris*), blue (*A. evansii*), green (*T. aromatica*) and pink (*Geobacter* species). Enzymatic steps include a cyclohexa-1,5-dienecarbonyl-CoA hydratase (encoded by *bamR/bzdW/dch*), 6-hydroxycyclohex-1-ene-1-carbonyl-CoA dehydrogenase (*bamQ/bzdX/had*), and 6-oxocyclohex-1-ene-carbonyl-CoA hydrolase (*bamA/bzdY/oah*). In *R. palustris*, the downstream reactions are carried out by a cyclohex-1-ene-1-carboxyl-CoA hydratase, a 2-hydroxycyclohexanecarboxyl-CoA dehydrogenase, and a 2-ketocyclohexanecarboxyl-CoA hydrolase, encoded by *badK*, *badH*, and *badI*, respectively. Dashed arrows indicate hypothetical enzymatic reactions. Sequential arrows indicate successive enzymatic transformations

Anaerobic catabolic pathways for the degradation of MAHs

In anoxic environments, bacteria metabolize aromatic compounds by replacing O_2 with alternative electron acceptors, such as nitrate (NO_3^-), sulfate (SO_4^{2-}), carbon dioxide (CO_2), and ferric iron (Fe^{3+}) (Pandolfo et al. 2023). Representative bacteria anaerobically degrading AHs include the denitrifiers *Thauera aromatica* and *Azoarcus evansii*, as well as the photoheterotroph *Rhodopseudomonas palustris* and the strict anaerobe *Geobacter metallireducens* (Porter and Young 2014). Like their aerobic counterparts, anaerobic microorganisms funnel AHs into central intermediates, often as Coenzyme-A (CoA) thioesters, either directly or following substrate activation. Most monoaromatic compounds are funnelled to the benzoyl-CoA central intermediate (Porter and Young 2014; Phale et al. 2020). However, other key intermediates, such as resorcinol, phloroglucinol, and hydroxyhydroquinone, have also been identified (Durante-Rodríguez et al. 2018; Boll et al. 2020).

Several biochemical strategies have been described for the activation of the aromatic ring, including fumarate addition, phosphorylation, O_2-independent hydroxylation, methylation, direct carboxylation, and ATP-dependent CoA ligation (Ladino-Orjuela et al. 2016; Hernández-Ospina et al. 2024). In contrast, the downstream degradation


(a) Upper pathway
Benzene
Methylation
Methylated aromatic (toluene)
Arylalkyl succinates (e.g., 4-hydroxybenzylsuccinate)
Fumarate addition
Xylenes (o-, m-, p-)
Cresols (o-, m-, p-)
Toluene
Phenol
Phosphorylation
Aromatic phosphate ester (phenylphosphate)
Aromatic carboxylates (e.g., benzoate)
Carboxylation
Benzene
Phenol
Phenylacetate
CoA-ligation
Aryl-CoA thioesters (e.g., phenylacetyl-CoA)
Benzoyl-CoA
Benzyl alcohol and phenolic derivatives (e.g. 1-phenylethanol)
O2-independent hydroxylation
Ethylbenzene
Benzene
Toluene
Anthranilate
Benzoate
Phtalate
4-hydroxybenzoate
3-hydroxybenzoate
ATP-dependent Class I Benzoyl-CoA reductase
badDEFG (Rhodopseudomonas palustris)
bzdNOPQ (Azoarcus evansii)
bcrCBAD (Thauera aromatica)
ATP-independent Class II Benzoyl-CoA reductase
bamBCDEFGHI genes (Geobacter species)
(b) Lower pathway
2[H]
Cyclohex-1-ene-1-carboxyl-CoA
Cyclohex-1,5-diene-1-carboxyl-CoA
H2O
bamR
bzdW
dch
6-Hydroxycyclohex-1-ene-1-carboxyl-CoA
H2O
badK
2-Hydroxy-cyclohexane-1-carboxyl-CoA
bamQ
bzdX
had
NAD+
NADH + H+
Modified β-oxidation reactions
NAD+
NADH + H+
badH
2-Ketocyclohexane-1-carboxyl-CoA
6-Oxocyclohex-1-ene-1-carboxyl-CoA
H2O
badI
bamA
bzdY
oah
H2O
Pimelyl-CoA
3-Hydroxypimelyl-CoA
3 Acetyl-CoA + CO2

pathway for the benzoyl-CoA appears to be more conserved (Fig. 2).

Benzoate provides a model substrate for studying the benzoyl-CoA pathway (Boll et al. 2020). In the upper pathway, benzoate is activated to benzoyl-CoA by benzoate-CoA ligase (BCL). This enzyme belongs to the aryl-CoA ligases/synthetases (ACL) family, a broad group of enzymes that catalyze the ATP-dependent thioesterification of various monoaromatic acids. Genes encoding BCL have been identified in various anaerobes and are named differently across studied organisms, such as *R. palustris* (*badA*), *T. aromatica* (*bclA*), *Azoarcus* spp. (*bzdA*), and *G. metallireducens* (*bamY*) (Carmona et al. 2009). Interestingly, other ACLs have also been shown to recognize benzoate as a substrate for activation, including 4-chlorobenzoate-CoA ligase, 2-aminobenzoate-CoA ligase, and 4-hydroxybenzoate-CoA ligase (Arnold et al. 2021).

In the lower pathway, benzoyl-CoA undergoes reductive dearomatization catalyzed by benzoyl-CoA reductase (BCR), yielding cyclohexa-1,5-diene-1-carboxyl-CoA (dienoyl-CoA). The complete degradation of this compound then proceeds via β-oxidation-like reactions to yield acetyl-CoA and CO_2 (Boll et al. 2020). Facultative and obligate anaerobes use three enzymatic steps to convert dienoyl-CoA to hydroxypimelyl-CoA. The exception is *R. palustris*, where benzoyl-CoA is reduced twice to cyclohex-1-ene-1-carboxyl-CoA and further metabolized to pimelyl-CoA through a modified β-oxidation pathway (Schmid et al. 2015; Durante-Rodríguez et al. 2018). At present, it remains unclear whether additional variants of the benzoyl-CoA degradation pathway exist in other bacteria.

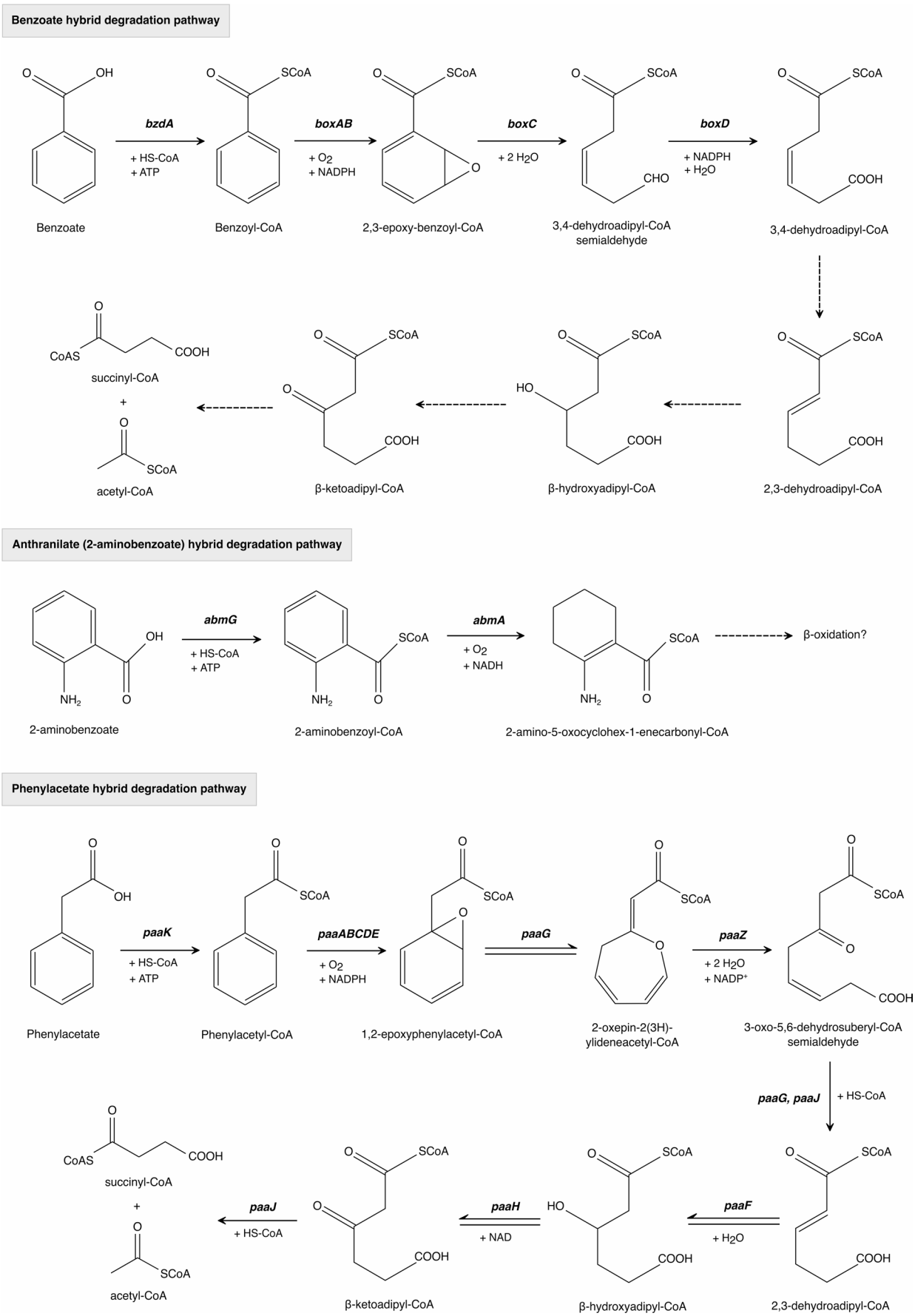
While analogous BCLs mediate benzoate activation, phylogenetic studies reveal that the subsequent dearomatization step is catalyzed by two distinct classes of BCRs, suggesting independent evolutionary origins in nature. Class I BCRs catalyze ATP-dependent, irreversible reactions and were long thought to occur exclusively in facultative anaerobes. However, recent genomic and biochemical studies revealed the presence of *Azoarcus*-type class I BCRs (*bzdNOPQ*) in the strictly anaerobic archaeon *Ferroglobus placidus*, thereby extending their distribution beyond facultative bacteria. In contrast, class II BCRs are ATP-independent, reversible enzymes widely distributed among strictly anaerobic bacteria, such as *Geobacter* and *Syntrophus* (Schmid et al. 2015; Tiedt et al. 2018; Boll et al. 2020). Despite their mechanistic differences, both classes share extreme sensitivity to oxygen, which accounts for their absence in aerobic organisms. The genes encoding BCR subunits are arranged into clusters, such as *bcrCBAD* (*T. aromatica*) and *bamBCDEFGHI* (*G. metallireducens*) (Boll et al. 2014), ensuring coordinate expression of the multimeric enzyme complex.

Fig. 3 Aerobic hybrid pathways for benzoate, 2-aminobenzoate (anthranilate) and phenylacetate degradation in ***Azoarcus*** **sp.** Aromatic compounds are initially activated to benzoyl-CoA through ATP-dependent Aryl-CoA ligase, a mechanism typical of anaerobic pathways. However, the subsequent ring dearomatization proceeds through an oxygen-dependent epoxidation followed by hydrolytic ring opening, distinct from both classic aerobic (dioxygenase-based) and strictly anaerobic (reductive) pathways. Genes: *bzdA*, Benzoate-CoA ligase; *boxAB*, benzoyl-CoA 2,3-epoxidase; *boxC*, benzoyl-CoA-dihydrodiol lyase; *boxD*, 3,4-dehydroadipyl-CoA semialdehyde dehydrogenase; *abmG*, 2-aminobenzoate-CoA ligase; *abmA*, 2-aminobenzoyl-CoA monooxygenase; *paaK*, phenylacetate-CoA ligase; *paaABCDE*, ring-1,2-phenylacetyl-CoA epoxidase; *paaG*, ring-1,2-epoxy-phenylacetate-CoA isomerase; *paaZ*, oxepin-CoA hydrolase/3-oxo-5,6-dehydrosuberyl-CoA semialdehyde dehydrogenase; *paaJ*, 3-oxoadipyl-CoA/3-oxo-5,6-dehydrosuberyl-CoA thiolase; *paaF*, 2,3-dehydroadipyl-CoA hydratase; *paaH*, 3-hydroxyadipyl-CoA dehydrogenase. Dashed arrows indicate hypothetical enzymatic reactions. Question marks denote an entirely speculative step

Hybrid catabolic pathways for the degradation of AHs

A third degradation strategy for mineralizing benzoate and other aromatic compounds, such as phenylacetate (Phe) and anthranilate, has been experimentally identified in a limited number of bacterial species (Díaz et al. 2013; Arora 2015). These novel pathways, termed *hybrid pathways*, involve an initial activation of the aromatic ring through CoA thioesterification, followed by epoxidation, and an oxygen-independent hydrolytic ring cleavage (Fig. 3) (Fuchs et al. 2011). The *box pathway* from *A. evansii* was the first hybrid strategy identified (Gescher et al. 2002; Godínez-Pérez et al. 2024). This pathway begins with the activation of benzoate to benzoyl-CoA by a BCL that differs from its anaerobic counterpart in both molecular mass and chromosomal gene location (Arnold et al. 2021). Benzoyl-CoA is then subjected to oxygen-dependent epoxidation by the multicomponent monooxygenase BoxAB, followed by hydrolytic ring cleavage that bypasses the need for dioxygenases typically required in aerobic degradation. Subsequent steps involve oxidation, isomerization, and hydroxylation reactions that lead to the yet-undetected but likely intermediate β-ketoadipyl-CoA.

In silico analysis revealed that *box* genes are present in many α- and β-proteobacteria and some δ-proteobacteria (Fuchs et al. 2011). Furthermore, it has been reported that several strains harbor both the classical anaerobic and hybrid pathways for benzoate degradation, which are expressed depending on oxygen availability during growth (Valderrama et al. 2012). The utilization of CoA thioesters as initial intermediates has been suggested to offer advantages to microaerophilic and facultative anaerobes by allowing them to unify the uptake mechanism of aromatic compounds (Kawaguchi et al. 2006). This is particularly beneficial under fluctuating oxygen conditions, as a decrease below


Benzoate hybrid degradation pathway
bzdA
+ HS-CoA
+ ATP
boxAB
+ O2
+ NADPH
boxC
+ 2 H2O
boxD
+ NADPH
+ H2O
Benzoate
Benzoyl-CoA
2,3-epoxy-benzoyl-CoA
3,4-dehydroadipyl-CoA semialdehyde
3,4-dehydroadipyl-CoA
2,3-dehydroadipyl-CoA
β-hydroxyadipyl-CoA
β-ketoadipyl-CoA
succinyl-CoA
acetyl-CoA
Anthranilate (2-aminobenzoate) hybrid degradation pathway
abmG
+ HS-CoA
+ ATP
abmA
+ O2
+ NADH
β-oxidation?
2-aminobenzoate
2-aminobenzoyl-CoA
2-amino-5-oxocyclohex-1-enecarbonyl-CoA
Phenylacetate hybrid degradation pathway
paaK
+ HS-CoA
+ ATP
paaABCDE
+ O2
+ NADPH
paaG
paaZ
+ 2 H2O
+ NADP+
Phenylacetate
Phenylacetyl-CoA
1,2-epoxyphenylacetyl-CoA
2-oxepin-2(3H)-ylideneacetyl-CoA
3-oxo-5,6-dehydrosuberyl-CoA semialdehyde
paaG, paaJ
+ HS-CoA
paaF
+ H2O
paaH
+ NAD
paaJ
+ HS-CoA
2,3-dehydroadipyl-CoA
β-hydroxyadipyl-CoA
β-ketoadipyl-CoA
succinyl-CoA
acetyl-CoA

a critical oxygen threshold would permit a rapid and energetically efficient transition between aerobic and anaerobic degradation routes.

Recently, there has been increasing interest in characterizing ACL enzymes, as they are essential for generating the central benzoyl-CoA intermediate. However, biochemical data for most ACL enzymes and associated pathways remain limited. To date, only a few ACLs have been purified from bacteria capable of growing aerobically or anaerobically on aromatic substrates (Arnold et al. 2021). These enzymes exhibit broad substrate specificity, recognizing and activating benzoate and its structural analogs such as phenylacetate, 2-, 3-, and 4-fluorobenzoate, 2-aminobenzoate, and 3- and 4-hydroxybenzoate, into their corresponding CoA-thioesters. Structural and kinetic characterizations have so far been conducted only for the CoA ligases of benzoate, phenylacetate, anthranilate, and 4-chlorobenzoate, providing valuable insights into their catalytic mechanisms and the key residues determining substrate specificity (Bains and Boulanger 2007; Erb et al. 2008; Gulick et al. 2004).

Microbial strategies for PAHs degradation

The degradation rate of PAHs is primarily influenced by physicochemical factors, including the number of aromatic rings, temperature, and water solubility. LMW PAHs, being more volatile and water-soluble, are more readily degraded by bacteria, whereas HMW PAHs pose a greater challenge due to their low solubility and limited bioavailability. Additionally, bacteria might lack the specific enzymes needed to initiate the breakdown of these complex molecules. Therefore, the complete mineralization of PAHs in natural environments often depends on the synergistic activity of diverse microorganisms. In microbial consortia, fungi and algae can initiate partial oxidation reactions. These include peroxidase- or laccase-mediated transformations into quinones or phthalates, which increase the bioavailability of otherwise recalcitrant PAHs (Hoque et al. 2023; Mou et al. 2023). These intermediates can subsequently be metabolized by bacteria through aerobic or anaerobic routes, ultimately leading to ring fission and mineralization to CO_2. Figure 4 summarizes the primary microbial pathways involved in the degradation of PAHs under both aerobic and anaerobic conditions, as well as the auxiliary oxidative contributions of fungi and algae that enhance the bioavailability of PAHs.

Similar to monoaromatic compounds, the aerobic degradation of PAHs in bacteria typically begins with a hydroxylation step catalyzed by ring-hydroxylating dioxygenases (RHDs), producing cis-dihydrodiols that are then oxidized to catechols. However, some *Mycobacterium* strains can mineralize PAHs through cytochrome P450 monooxygenase activity, yielding trans-dihydrodiols as intermediates (Cao et al. 2022). Aerobic degradation of PAHs is well documented for model substrates such as naphthalene, anthracene, and phenanthrene. Among the best-characterized systems is the *nah* operon in *P. putida* G7 (plasmid NAH7), which encodes enzymes for the conversion of naphthalene into salicylate (upper pathway) and subsequently into pyruvate and acetaldehyde (lower pathway) (Haritash and Kaushik 2009).

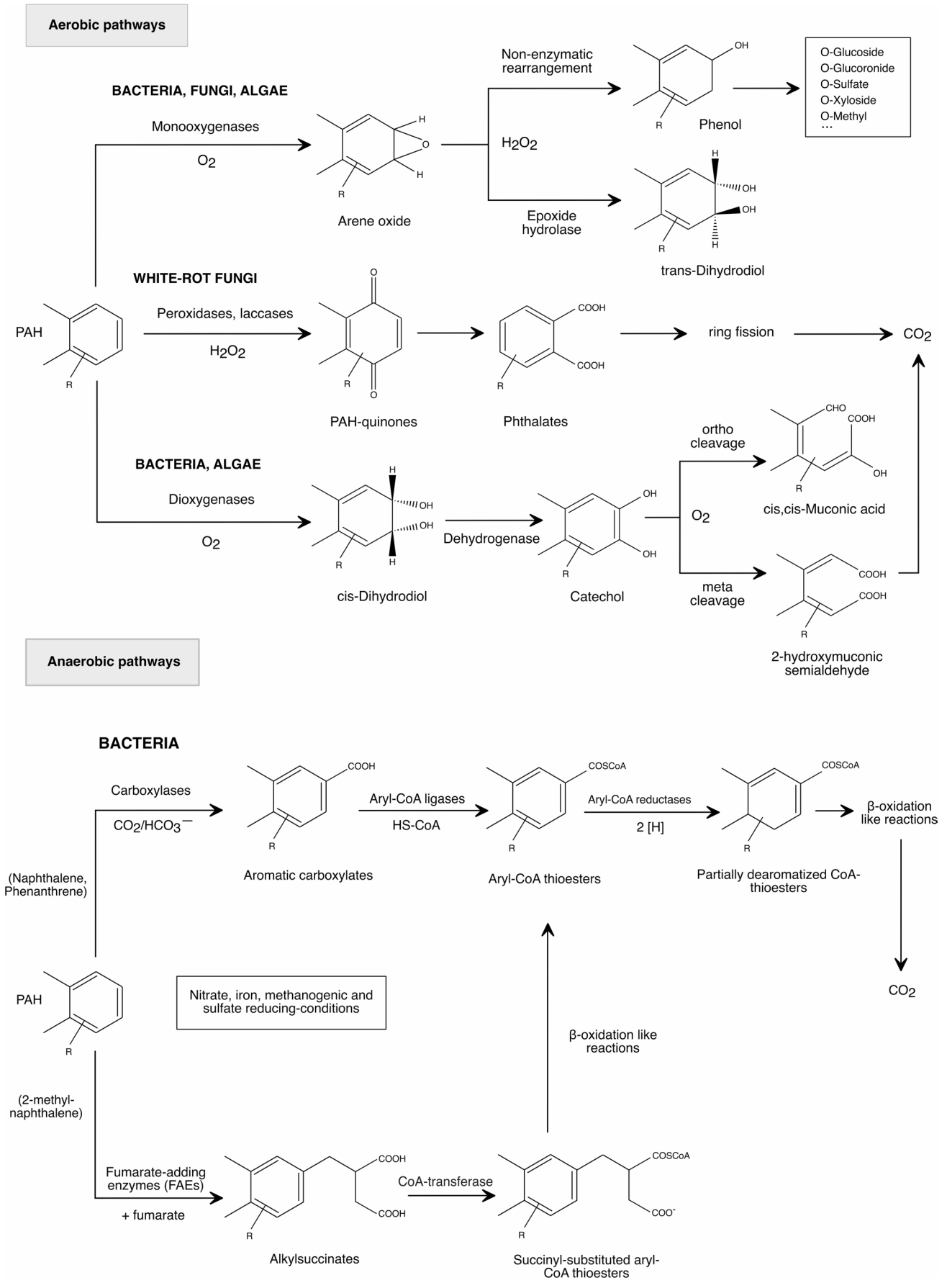
Fig. 4 Degradation of PAHs by microorganisms. In aerobic microorganisms (bacteria, fungi, algae), PAHs are initially oxidized by monooxygenases or dioxygenases, yielding arene oxides or cis-dihydrodiols, which are further transformed to catechols or phenols. White-rot fungi employ peroxidases and laccases, producing PAH-quinones and phthalates. These intermediates undergo subsequent transformations, including dehydrogenation and ring-cleavage reactions (ortho or meta), ultimately leading to mineralization into CO_2. In anaerobic bacteria, LMW-PAHs are activated by carboxylation, CoA ligation, or fumarate addition. The resulting intermediates are reduced by aryl-CoA reductases and further metabolized through β-oxidation-like reactions

Whereas fungi and algae can effectively oxidize PAHs aerobically, bacteria display a remarkable ability to degrade PAHs anaerobically in many natural and polluted sites. Nevertheless, the underlying mechanisms of anaerobic PAHs degradation remain poorly characterized. To date, anaerobic degradation has been partially elucidated for LMW-PAHs such as naphthalene, 2-methylnaphthalene, and phenanthrene. In these pathways, strictly and facultative anaerobes initiate aromatic ring activation using some biochemical strategies described for monoaromatics. Under sulfate-reducing conditions, 2-methyl-naphthalene is activated by fumarate addition, similar to anaerobic toluene degradation. The unsubstituted PAHs naphthalene and phenanthrene are activated through carboxylation to 2-naphthoate/2-phenanthroate by UbiD-like carboxylases, followed by CoA-thioesters formation through ACLs (Samak et al. 2025). Subsequent dearomatization proceeds via ATP-independent reductions catalyzed by a novel class of Aryl-CoA reductases (ACRs).

Biotechnological potential of AHs degradation

Beyond expanding ecological and biochemical knowledge, elucidating microbial pathways for AHs degradation provides new opportunities for biotechnological applications.

Field studies have demonstrated enhanced in situ bioremediation through bioaugmentation and stimulation of native bacterial degraders. *Pseudomonas putida* bioaugmentation enhanced treatment of phenolic landfill leachate in sequencing batch reactors (Michalska et al. 2020), and immobilized *Pseudomonas* spp. removed BTEX from contaminated groundwater in a fibrous-bed bioreactor (Shim and Yang 2002). Anaerobic degraders expand their applicability to oxygen-limited sites (e.g., sediments, aquifers),


Aerobic pathways
BACTERIA, FUNGI, ALGAE
Monooxygenases
O2
Arene oxide
Non-enzymatic rearrangement
H2O2
Epoxide hydrolase
Phenol
O-Glucoside
O-Glucoronide
O-Sulfate
O-Xyloside
O-Methyl
trans-Dihydrodiol
WHITE-ROT FUNGI
PAH
Peroxidases, laccases
H2O2
PAH-quinones
Phthalates
ring fission
CO2
BACTERIA, ALGAE
Dioxygenases
O2
cis-Dihydrodiol
Dehydrogenase
Catechol
ortho cleavage
meta cleavage
cis,cis-Muconic acid
2-hydroxymuconic semialdehyde
Anaerobic pathways
BACTERIA
Carboxylases
CO2/HCO3−
Aromatic carboxylates
Aryl-CoA ligases
HS-CoA
Aryl-CoA thioesters
Aryl-CoA reductases
2 [H]
Partially dearomatized CoA-thioesters
β-oxidation like reactions
CO2
(Naphthalene, Phenanthrene)
PAH
Nitrate, iron, methanogenic and sulfate reducing-conditions
(2-methyl-naphthalene)
Fumarate-adding enzymes (FAEs)
+ fumarate
Alkylsuccinates
CoA-transferase
Succinyl-substituted aryl-CoA thioesters
β-oxidation like reactions

while consortia can outperform single strains (Forján et al. 2020; Hoque et al. 2023).

Hydrocarbon-degrading enzymes have also found applications in industrial biocatalysis. For instance, styrene monooxygenase has been employed for the enantioselective epoxidation of styrene to produce (S)-styrene oxide at pilot scale, a valuable precursor for pharmaceuticals and fragrances (Panke et al. 2002). Toluene dioxygenase has been engineered to enhance its activity for the dihydroxylation of bulky and ester-functionalized aromatics (Osifalujo et al. 2022). Currently, oxygenases involved in central pathways, such as catechol 1,2-dioxygenase, are now viewed less for their ecological role and more as strategic biocatalysts enabling renewable routes to industrial monomers like adipic acid (Kruyer et al. 2020). Synthetic biology has further expanded the use of aromatic-degrading enzymes by integrating them into heterologous pathways to drive the sustainable production of value-added compounds (Curran et al. 2013; Han et al. 2015).

Omics-driven insights into AH degradation pathways

In recent years, the integration of omics technologies and bioinformatic tools has greatly advanced the study of microbial catabolism. A key driver of this paradigm shift has been the advancement of sequencing technologies. Sequencing strategies have evolved from early chemical methods and manual gel-based techniques to automated fluorescent methods, culminating in modern high-throughput platforms, including next-generation sequencing (NGS) and long-read technologies. These approaches now facilitate the rapid and large-scale characterization of microbial genomes, including those of uncultured or low-abundance organisms. At the same time, multi-omics data provide complementary insights into gene expression, protein function, and metabolic activity. Several databases and predictive tools/resources currently assist the processing, integration, and interpretation of these large-scale datasets (Fig. 5).

Today, the increased availability of sequence genomes, combined with core bioinformatic tools, such as BLAST for rapid sequence alignment (Altschup et al. 1990), MMseqs2 for sensitive clustering of homologs (Steinegger and Söding 2017), MEME for de novo motif discovery (Bailey and Elkan 1994), and HMMER for profile-based homology detection (Potter et al. 2018), enables the functional prediction of previously uncharacterized enzymes involved in AHs degradation across diverse taxa. To enhance the accuracy and scalability of these predictions, protein-family models built from multiple sequence alignments and profile Hidden Markov Models (HMMs) are curated in specialized databases such as Pfam (Mistry et al., 2021). These resources serve as components for annotation pipelines like RAST/RAS-Ttk (Brettin et al. 2015) and eggNOG-mapper-v2 (Cantalapiedra et al., 2021), which automate functional annotation across genomes or metagenomes. Such annotations are further enriched by cross-referencing with biochemical and enzymatic databases, including KEGG (Kanehisa et al., 2016), MetaCyc (Krieger et al. 2004), BRENDA (Chang et al. 2021), UniProt (Bateman et al. 2023), PDB (Berman et al. 2002), and PubChem (Kim et al. 2021), which provide curated insights into enzyme function, metabolic reactions, and chemical structures.

Together, these resources have consolidated biochemical, enzymatic, and structural information, enabling accurate function assignment, genome-wide identification of candidate AHs–degrading enzymes, and the reconstruction of complete catabolic pathways from genomic and metagenomic data (Fig. 6).

Comparative genomics

Comparative genomics is the systematic comparison of biological information derived from whole-genome sequences (de Crécy-Lagard and Hanson 2013). This approach supports gene annotation, metabolic potential prediction, and the reconstruction of metabolic and regulatory networks associated with AHs degradation (Suvorova and Gelfand 2019; Khot et al. 2022). Such efforts have led to the discovery of genes encoding novel AHs-degrading enzymes. In *Desulfatiglans* TRIP_1, Kraiselburd et al. (2019) combined MicroScope, KEGG, MetaCyc, CheckM, and homology searches with transcriptomic and proteomic data to propose a phenanthroate-CoA ligase and UbiD-like carboxylases involved in phenanthrene degradation. Building on these predictions, Kaplieva-Dudek et al. (2024) later purified and characterized the 2-phenanthroate-CoA ligase, providing biochemical validation of its activity.

Broader comparative genomics studies have shown the extensive genetic potential for aromatic catabolism across diverse bacteria. For example, a comparative genomics study in *Cupriavidus necator* JMP134 enabled the prediction of numerous catabolic pathways through genome sequencing, in silico annotation, gene-context analysis, and phylogenetic reconstruction. These predictions were experimentally validated, as the strain was shown to grow on 60 aromatic substrates, metabolized through the β-ketoadipate, gentisate, homogentisate, phenylacetyl-CoA, and benzoyl-CoA pathways (Pérez-Pantoja et al. 2008).

Genomic analyses are now extending this approach beyond single-organism genomes. A survey of 80 *Burkholderiales* genomes, using BLASTP searches and phylogenetic reconstruction of 48 oxygenase markers, revealed nearly all

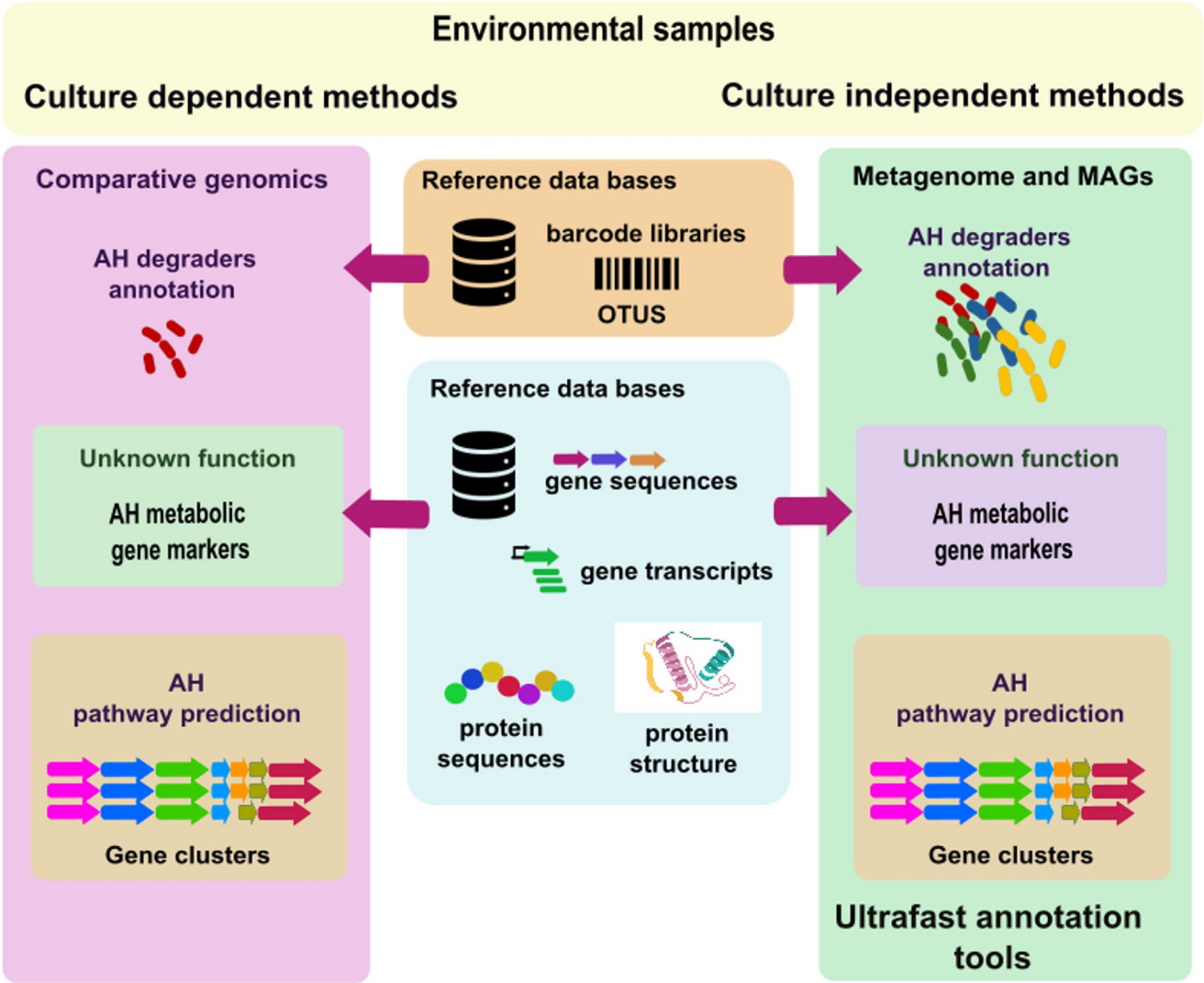


Fig. 5 Strategies for exploring aromatic hydrocarbon degradation from environmental samples. Culture-dependent approaches and culture-independent methods, enable degrader annotation, identification of metabolic gene markers, and pathway prediction from gene clusters. Both rely on reference databases that integrate barcode libraries, gene and protein sequences, transcripts, and protein structures

central ring-cleavage pathways (Pérez-Pantoja et al. 2012). More recently, genome sequencing and annotation (RAS-Ttk, DRAM) of 22 bacterial strains from contaminated soils identified hundreds of genes for the degradation of benzoate, gentisate, homogentisate, protocatechuate, BTEX, and LMW-PAHs (Hossain et al. 2024).

Metagenomics and metagenome-assembled genomes

Metagenomics enables the study of uncultured microbes directly from environmental DNA, revealing both community composition and the metabolic potential of complex microbial consortia involved in AHs biodegradation. Functional annotation of metagenomes typically relies on optimized comparative-genomics tools (Fig. 5). Ultrafast homology searches against reference protein databases such as UniProt and NCBI RefSeq (O'Leary et al. 2016) are commonly performed with DIAMOND (Buchfink et al. 2021), while orthology-based tools like eggNOG-mapper v2 are now standard for large-scale functional inference in environmental datasets. Additionally, the recovery of metagenome-assembled genomes (MAGs) yields near-complete or draft genomes reconstructed from community datasets via assembly and binning (Yang et al. 2021). Through MAGs, we can better understand microbial diversity, reconstruct metabolism, and study AH degradation in natural and polluted environments, from deep-sea sediments to polluted soils.

Case studies underscore the shift from tools to discoveries. Stable isotope probing (SIP) coupled with shotgun metagenomics identified uncultured *Rhodocyclaceae* as

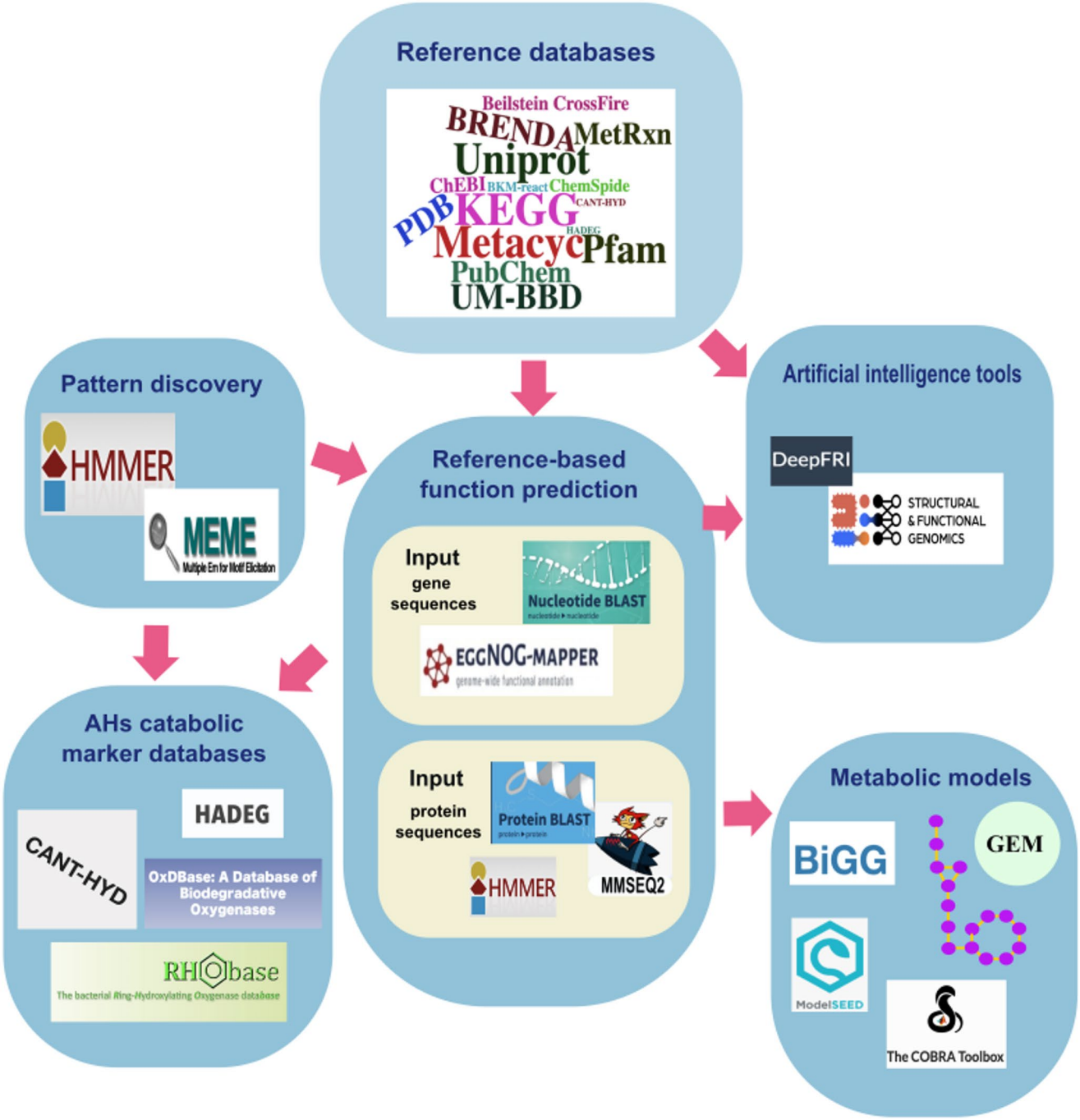


Fig. 6 Bioinformatic tools and resources for the functional prediction of genes, enzymes, and pathways involved in AHs degradation. Reference databases (e.g., KEGG, UniProt, Pfam, MetaCyc) provide essential information on genes, proteins, and metabolic pathways. Pattern discovery tools such as HMMER and MEME are used to detect conserved domains and sequence motifs, while specialized AH catabolic marker databases (CANT-HYD, HADEG, OxDBase, RHObase) compile curated data on genes and enzymes directly involved in AH degradation. Gene and protein sequences can be analyzed through reference-based function prediction tools (BLAST, eggNOG-mapper, MMSEQ2, HMMER), with annotations further improved using artificial intelligence approaches (e.g., DeepFRI). Finally, predicted functions and pathways can be incorporated into genome-scale metabolic models (BiGG, ModelSEED, GEMs, COBRA Toolbox) to reconstruct metabolic networks and evaluate the ecological or biotechnological roles of AH degraders

dominant phenanthrene degraders in contaminated soils, enabling the cloning of novel RHDs with distinct substrate specificities. MAGs recovered from a refinery wastewater treatment plant under nitrogen limitation showed that *Bradyrhizobiaceae* encode nitrogen fixation pathways and at least 14 oxygenases for AH utilization (Tikariha and Purohit 2019). More recently, Artificial Intelligence (AI)-guided metagenome mining, which combined HMM profiles, structural modeling, and functional validation, uncovered sequence-divergent dioxygenases/peroxidases that were missed by homology searches (Nagy et al. 2024).

As a natural extension of these findings, specialized resources such as HADEG, CANT-HYD, RHObase, and OxDBase have emerged to support hydrocarbon degradation research. HADEG compiles experimentally validated genes involved in aerobic degradation, serving as a reference framework for reliable annotation (Chakraborty et al. 2014; Rojas-Vargas et al. 2023). CANT-HYD provides a set of 37 HMMs designed for the identification and annotation of marker genes involved in anaerobic and aerobic degradation, making it suitable for large-scale genome and metagenome annotation (Khot et al. 2022). In contrast, RHObase and OxDBase focus on oxygenase enzymes, providing sequence, structural, and functional information, along with datasets that can be directly used to perform BLAST searches for functional prediction (Arora et al. 2009; Chakraborty et al. 2014). While these resources represent valuable efforts, the number of bioinformatic tools specifically designed for AHs degradation research remains very limited, and most of them are focused on aerobic rather than anaerobic processes. This highlights the need for resources that address the full range of microbial catabolic strategies.

Multi-omics integration

While genomics and metagenomics reveal the genetic potential of microbial communities, they provide only a static inventory of possible functions. Capturing the active processes that determine AHs degradation requires additional omics layers such as transcriptomics, proteomics, and metabolomics. These approaches enable the transition from prediction to direct observation of regulation, expression, and metabolic outcomes in situ.

Transcriptomics describes the dynamic gene expression under specific environmental conditions. For example, RNA-Seq studies have shown the induction of genes encoding RHDs and peripheral catabolic enzymes when bacteria are exposed to AHs, thereby validating predictions derived from genomic data (Luo et al. 2014; Peng et al. 2020; Zampolli et al. 2020). Moreover, transcriptomic profiling allows the discovery of novel regulators and transcriptional networks that fine-tune catabolic pathways in response to substrate availability or stress (Martinez-Varela et al. 2023). This approach has also been highlighted in the context of synthetic biology, where transcriptomics is considered a key step to characterize hydrocarbon-responsive regulatory systems and to repurpose them as biosensors (Moratti et al. 2022).

Proteomics complements transcriptomics approaches by giving direct information about enzymes rather than RNA. Techniques such as Liquid Chromatography–Tandem Mass Spectrometry (LC–MS/MS), have been employed to quantify the expression of degrading-enzymes such as oxygenases, CoA ligases, and reductases. For example, Heintz et al. (2009) used differential membrane proteomics to reveal the first ATP-independent Class II BCR in *Geobacter metallireducens*. Similarly, proteomic profiling of *Rhodococcus* sp. TFB indicated induction of enzymes specific to phthalate and naphthalene metabolism, enabling the reconstruction of a complete phthalate degradation pathway (Tomás-Gallardo et al. 2006). A proteogenomic study in *Burkholderia* sp. K24 confirmed the coexistence of multiple independent pathways for aniline, benzoate, and toluene degradation (Lee et al. 2016). More recently, proteomic surveys have emphasized their value in detecting post-translational modifications, enzyme isoforms, and unexpected metabolic interactions, thus providing a way to complement transcriptomic predictions (Kim et al. 2009).

Metabolomics adds the downstream dimension by profiling small molecules and pathway intermediates. Through techniques such as Gas Chromatography–Mass Spectrometry (GC–MS) or Nuclear Magnetic Resonance (NMR), metabolomics allows detection of diagnostic intermediates. For instance, GC–MS has been employed to identify catechol and cis, cis-muconate during phenol degradation, thereby validating the ortho-cleavage pathway in yeast cells (Filipowicz et al. 2020), and to detect deuterated phenol as the initial intermediate in benzene degradation by halophilic *Arhodomonas* sp., confirming hydroxylation as the entry step (Dalvi et al. 2012). Complementary targeted metabolomics of CoA-thioesters using LC–MS/MS has also uncovered activated intermediates such as halobenzoyl-CoA and benzoyl-CoA in anaerobic degraders, providing chemical evidence for pathway variants (Kuntze et al. 2011; Cakić et al. 2021).

When integrated, these omics approaches create a multilayered systems view of AHs degradation. As such, multiomics integration bridges the gap between genetic potential and realized function.

Emerging approaches

AI and machine learning (ML) now enhance functional genomics by predicting enzyme functions and metabolic potential. DeepFRI (Gligorijević et al. 2021) infers function from sequence and structural features even in the absence of detectable homology. AntiSMASH and ML classifiers have predicted biosynthetic gene clusters across bacterial, fungal, and plant genomes within the ANL superfamily, which includes ACLs central to AHs degradation (Robinson et al. 2020). The ML-classified ANLs enabled phylogenetic reconstruction, suggesting ancient ANLs had active sites most similar to modern enzymes that use CoA-SH as the acceptor.

Genome-scale metabolic models (GEMs), via BiGG (Norsigian et al. 2020), ModelSEED (Seaver et al. 2021), MOST (Kelley et al. 2017), and the COBRA toolbox (Heirendt et al. 2019), integrate these predictions into networks, enabling in silico flux and pathway simulations. For example, the reconstruction of *Pseudomonas putida* KT2440 using COBRA enabled flux balance simulations that validated the catabolic pathway for toluene degradation (Nogales et al. 2008). Similarly, a curated GEM of *P. fluorescens* predicted catechol degradation through the β-ketoadipate pathway using RAST and ModelSEED (Huang and Lin 2020).

Despite their growing utility, both AI-based predictions and GEM reconstructions remain limited by annotation gaps, biased training datasets, and the lack of experimental validation. Addressing these challenges will require integrating curated multi-omics data, improving modeling algorithms, and incorporating biochemical evidence to fully exploit their potential in elucidating AHs degradation pathways.

Limitations, future perspectives, and conclusions

Omics and bioinformatics have deepened our understanding of the degradation of AHs, revealing new enzymes, extending known pathways to overlooked taxa, and uncovering alternative routes. Genomics and metagenomics are now essential for probing niches where cultured and uncultured microbes work together to degrade and mineralize AHs.

Despite these advances, our biochemical understanding of key anaerobic steps remains incomplete. For example, the proposed anaerobic benzene carboxylase (UbiD/UbiX-type) is supported by omics evidence yet remains only partially resolved biochemically. Likewise, the activation of naphthalene via carboxylation in the sulfate-reducing culture N47 has been mechanistically outlined, but the corresponding enzyme is still being consolidated. In the case of phenanthrene, downstream steps involving 2-phenanthroate-CoA ligase and 2-phenanthroyl-CoA reductase have been defined, yet the identity and diversity of the initial activating-carboxylase remain to be fully elucidated. These gaps highlight the importance of integrating omics predictions with experimental validation to unravel the full enzymatic potential of environmental microbes.

Another key limitation in AHs research is the shortage of specialized databases and tools for predicting catabolic genes and enzymes. Existing resources and reference databases are mostly restricted to aerobic pathways, focusing on oxygenases and other well-characterized enzymes (e.g., OxDBase, RHObase). Consequently, key anaerobic enzymes (e.g., aryl-CoA ligases, carboxylases, aryl-CoA reductases) remain poorly annotated and are rarely incorporated into predictive workflows. In parallel, reference datasets are dominated by model organisms like *Pseudomonas* and *Escherichia coli*, leaving uncultivated lineages underrepresented. As a result, catabolic strategies encoded in MAGs or single-cell genomes are often missed, and annotations in complex microbiomes can be incomplete or biased.

Finally, an additional challenge is the poor integration of omics layers that provide convergent evidence of AHs degradation pathways. Metagenomics, transcriptomics, proteomics, and metabolomics offer complementary insights, but they are rarely unified into predictive frameworks. Likewise, AI approaches that move beyond homology are seldom connected to experimental omics or metabolic models, which limits their ability to predict the function of divergent or poorly characterized enzyme families.

Addressing these limitations will require coordinated efforts to expand specialized databases, improve predictive frameworks, and link computational models more closely with ecological conditions. Progress will depend on incorporating enzymes reconstructed from MAGs and single-cell genomes, extending taxonomic coverage, and enriching catabolic annotations. Integrating genome-scale metabolic models with ecological simulations that account for oxygen gradients, nutrient levels, and pollutant concentrations could further bridge the gap between laboratory predictions and field conditions. Such advances will enhance prediction accuracy, facilitate the discovery of key microbial degraders and enzymes, and ultimately unlock the full bioremediation potential of microbial communities.

Abbreviations

AHs	Aromatic hydrocarbons
MAHs	Monocyclic aromatic hydrocarbons
PAHs	Polycyclic aromatic hydrocarbons
LMW PAHs	Low molecular weight PAHs
HMW PAHs	High molecular weight PAHs
TCA	Tricarboxylic acid

RCDs	Ring-cleaving dioxygenases
RHDs	Ring-hydroxylating dioxygenases
β-KAP	β-ketoadipate pathway
Edos	Extradiol dioxygenases
VOC	Vicinal oxygen-chelate
CoA	Coenzyme-A
BCL	Benzoate-CoA ligase
ACL	Aryl-CoA ligase/synthetase
BCR	Benzoyl-CoA reductase
Phe	Phenanthrene
HMMs	Hidden Markov Models
NGS	Next-generation sequencing
MAGs	Metagenome-assembled genomes
SIP	Stable isotope probing
GEMs	Genome-scale metabolic models
AI	Artificial Intelligence
ML	Machine learning
GC–MS	Gas Chromatography–Mass Spectrometry
NMR	Nuclear Magnetic Resonance
LC–MS/MS	Techniques such as Liquid Chromatography–Tandem Mass Spectrometry

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Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

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