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专论与综述

微生物降解石油烃的功能基因研究进展

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摘 要: 微生物对石油烃的降解在自然衰减去除土壤和地下水石油烃污染的过程中发挥了重要作用。微生物通过其产生的一系列酶来利用和降解这类有机污染物，其中，编码关键降解酶的基因称为功能基因。功能基因可作为生物标志物用于分析环境中石油烃降解基因的多样性。因此，研究石油降解功能基因是分析土著微生物群落多样性、评价自然衰减潜力与构建基因工程菌的重要基础。本文主要介绍了烷烃和芳香烃在有氧和无氧条件下的微生物降解途径，重点总结了烷烃和芳香烃降解的主要功能基因及其作用，包括参与羟化作用的单加氧酶和双加氧酶基因、延胡索酸加成反应的琥珀酸合酶基因以及中心中间产物的降解酶基因等。

关键词: 石油烃，自然衰减，微生物降解，降解机制，功能基因

Function genes in microorganisms capable of degrading petroleum hydrocarbon

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Abstract: Microorganisms play an important role in the natural attenuation process of petroleum hydrocarbons removal from the environment. Microorganisms can produce a series of enzymes to utilize and degrade these organic contaminants. The genes encoding the key enzymes in the hydrocarbon biodegradation pathway are called as function genes. Therefore, studying the function genes for petroleum hydrocarbon degradation is an important basis for analyzing the diversity of indigenous microbial

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communities, evaluating the natural decaying potential and constructing the genetic modified strains. This review introduces the aerobic and anaerobic microorganism for biodegradation of alkanes and aromatic compounds. The major function genes for the degradation are summarized, including genes encoding monooxygenases and dioxygenases.

Keywords: Petroleum hydrocarbon, Natural attenuation, Microbial degradation, Degradation mechanism, Function genes

随着工业与社会的快速发展，石油使用量与开采量剧增，导致环境的石油污染问题日益严重。石油成分复杂，其中富含的苯系物和多环芳烃物质结构稳定、不易挥发、难于降解、环境持留时间长、三致效应高[1]。如何有效防治石油烃的污染已成为科学研究与社会发展所重点关注的问题。环境中的石油烃可经历风化作用逐步分解，包括物理作用(扩散)、生理生化作用(蒸发，溶解，吸附，解析)、化学作用(光氧化，自氧化)及生物作用(植物和微生物的代谢)[2]。其中，微生物降解可将石油烃最终完全转化为无毒无害的 CO_2 和 H_2O，是石油烃自然衰减的主要作用。微生物降解石油烃是微生物利用石油烃作为其自身代谢的碳源和能源，进行转化后供其生长。影响微生物代谢石油烃的主要因素包括微生物自身特性(跨膜运输系统和产表面活性剂等)[3]、污染物的浓度和生化特性[4]以及电子受体(氧气、硝酸盐和 Fe^{3+} 等)[5]。环境条件如盐度、湿度、营养元素和土壤化学等会影响石油烃的性质或微生物的生长，也成为微生物降解石油烃的制约因素。石油污染发生后，大量微生物无法存活，土壤中微生物多样性和生物量显著降低，仅石油烃耐受菌和石油烃降解菌可存活下来并利用石油烃作为能量来源大量繁殖，其比例可由通常的 1%提高到 10%，在群落中占据主导地位[6-9]；随着时间的延长，石油烃整体毒性降低，微生物群落的数量和多样性又会增加[10]。

石油烃结构的复杂性决定了微生物降解菌的多样性。目前已发现数百种微生物，包括细菌、真菌和酵母菌可降解一种或多种石油烃类物质[11]。在石油烃物质存在时，微生物会表达特定功能基因，产生特异酶来进行石油烃代谢；功能基因的表达是微生物降解石油烃的关键。近年来，多组学技术包括基因组、转录组和蛋白质组等新兴技术快速发展，为微生物降解石油烃功能基因的快速测序和鉴定提供了有力的技术支撑。采用与已知功能基因进行序列比对、基因转录水平分析、功能酶表达水平等研究方法，可有效分析污染环境中降解微生物的功能基因多样性和丰度。Laczi 等[12]通过分析 *Rhodococcus erythropolis* PR4 的基因序列，鉴定了多个氧化酶基因，并利用转录组学技术分析和鉴定了降解柴油和其他石油烃的关键基因。红球菌是研究最多的石油烃降解微生物之一，近年来越来越多的研究利用多组学技术对该菌属的降解潜力展开研究[13]。功能基因不仅是研究石油降解微生物的群落结构特征、固有降解菌和降解潜力的基础，也是构建基因工程菌进行生物修复的依据。如将来源于 *Acidovorax* sp. CHX100 的环己烷降解功能基因：细胞色素 P450、铁氧还蛋白还原酶和铁氧还蛋白，在菌株 *Pseudomonas taiwanensis* VLB120 中进行过量表达，可极大地提高菌株对环己烷的氧化降解效能[14]。

石油烃污染源可分为石油开采的原油、炼化厂的减压渣油和加油站的成品油。尽管不同地区原油组成和性质各不相同，但是烷烃和芳香烃的比例高达 80%[15]。其中链烷烃在原油中的比例约为 50%−70%[16]，芳香烃约为 10%−30%；仅有极少数原油中链烷烃低于 10%−15%[16]。原油在炼化厂经过提炼后产生减压渣油，其主要组分为烷烃和芳香烃，其余组分为难降解的胶质和沥青质。

与原油相比，减压渣油中烷烃比例减少，芳香烃比例增加。我国部分地区的原油和减压渣油中烷烃和芳香烃比例见表 1[17-18]。成品油组分则几乎全部为烷烃。由此可见，烷烃和芳香烃是石油烃污染微生物降解与修复的主要目标污染物。微生物主要通过羟化作用、脱氢作用、过氧化作用等方式降解石油烃，包括好氧降解和厌氧降解。好氧降解是好氧微生物或兼性厌氧微生物以石油烃作为底物，分子氧作为最终电子受体，将石油烃转化为 H_2O、CO_2 和 NH_3 的过程[11]。厌氧降解则是厌氧微生物或兼性好氧微生物在无分子氧的条件下，以硝酸盐、硫酸盐、Mn^{4+}、Fe^{3+}、二氧化碳作为最终电子受体[19-20]，将石油烃有机物转化为 CH_4 等。好氧降解比厌氧降解的速率更快，所需时间更短[21]。本文系统梳理了微生物降解烷烃和芳香烃的好氧和厌氧降解途径，总结了有氧和无氧条件下微生物代谢烷烃和芳香烃的功能基因，为降解石油烃的微生物资源挖掘和基因工程菌的构建提供参考(表 2)。

表 1　原油和减压渣油中烷烃和芳香烃比例

Table 1　Chemical composition of crude oil and vacuum residuum (%)

油品 Oil	地点 Site	烷烃 Alkane	芳香烃 Aromatic hydrocarbon
原油 Crude oil	四川马岭 Maling, Sichuan	64.34	15.58
	四川华庆 Huaqing, Sichuan	54.29	10.72
减压渣油 Vacuum residuum	黑龙江大庆 Daqing, Heilongjiang	40.80	32.20
	新疆白克 Baike, Xinjiang	47.30	25.20
	新疆九区 Nine districts in Xinjiang	28.20	26.90
	辽宁欢喜岭 Huanxiling, Liaoning	28.70	35.00
	天津大港 Dagang, Tianjin	30.60	31.60
	中原 Zhongyuan	23.60	31.60

1　微生物好氧降解石油烃的关键酶与功能基因

1.1　烷烃的好氧微生物降解

直链烷烃的降解方式主要有末端氧化、次末端氧化、β-氧化和 ω-氧化[11,57-58]。在微生物好氧降解烷烃体系中，加氧酶的羟化作用是主要限速步骤。短链烷烃的单加氧酶主要为甲烷单加氧酶(methane monooxygenase，MMOs)，MMOs 分为可溶性甲烷单加氧酶(sMMO)和颗粒性甲烷单加氧酶(pMMO)，sMMO 能氧化 C_1−C_8 烷烃、卤代烃、烯烃、环烷烃和芳香烃，pMMO 能氧化 C_1−C_5 烷烃、卤代烃和烯烃[26,59]。AlkB 烷烃单加氧酶家族主要负责催化短链与中等链长烷烃的氧化[22]，该家族广泛存在于变形菌和放线菌中，是研究得最透彻的烷烃单加氧酶。*P. putida* GPo1 菌株中 AlkB 单加氧酶可氧化丙烷、丁烷和 C_5−C_{12} 短链烷烃；*alkB* 基因位于 OCT 质粒上的 *alkBFGHJKL* 基因簇，这一基因簇表达的一系列酶将烷烃转化为乙酰辅酶 A[22]；*alkST* 是这一基因簇的转录调控因子[59]。在 *P. putida* GPo1 菌株 AlkB 的同源蛋白中，很多能氧化链长超过 C_{10} 的烷烃。细胞色素 P450s 单加氧酶(cytochrome P450，CYPs)是一类硫醇盐蛋白超家族，可催化很多化合物的氧化反应。基于氨基酸序列相似性，CYPs 超家族可细分为不同的家族，其中氧化 C_6−C_{10} 烷烃的单加氧酶属于细菌 CYP153 家族[60]。CYP153 家族的底物除了烷烃，还包括柠檬烯、环己烯、苯乙烯和辛烯等[61-62]。

碳链超过 C_{18} 的烷烃以固体形式存在，通常将固体石蜡作为固体长链烷烃降解的研究对象。固体石蜡是烃类混合物，碳链长为 C_{20}−C_{40}[63]。Lazar 等[64]于 1999 年首次提出利用微生物降解固体长链烷烃中的石蜡组分。但是能以固体石蜡作为降解底物的微生物种类不多，目前发现的菌属有 *Pseudomonas*、*Bacillus*、*Acinetobacter*、*Gordonia*、*Geobacillus* 和 *Rhodococcus* 等[63]。微生物与固体烷烃的接触机制有两种：一种是与石蜡直接接触，

表 2 微生物降解石油烃的功能基因

Table 2 Function genes in the hydrocarbon degradation of microorganisms

降解基因 Degrading genes	降解底物 Substrates	参考菌株(基因序列号) Reference strains (GenBank accession number)	菌株来源 Sources	参考文献 References
alkB	C_5–C_{12} alkane	*Pseudomonas putida* GPo1 (CAB54064)	Machine shop cutting oil	[22]
a*lmA*	C_{10}–C_{40} alkane	*Acinetobacter* sp. DSM 17874 (ABQ18224)	Unsterilized oil	[23]
ladA	C_{15}–C_{36} alkane	*Geobacillus thermodenitrificans* NG80-2 (ABO68832)	Deep oil reservoir	[24]
alkM	C_{12}–C_{36} alkane	*Acinetobacter* sp. ADP1 (CAA05333)	Soil	[25]
Methane monooxygenase, MMOs	C_1–C_8 alkane, alkene, halogenated hydrocarbon, cycloalkane	*Methylocapsa* spp.	Forest soil, acid sphagnum peat bog, etc	[26]
Cytochrome P450 family, CYP153	Alkane, cycloalkane, aromatic hydrocarbon	*Dietzia* sp. DQ12-45-1b (AFY63004)	Oil production water of a deep subterranean oil reservoir	[27]
assA	C_{13}–C_{18} alkane, pentadecene, hexadecene	*Desulfatibacillum alkenivorans* AK-01 (ABH11461)	Oil-contaminated site	[28]
masD	C_6–C_8 alkane, alkylbenzene	*Aromatoleum* sp. HxN1 (CAO03074)	Sediment samples from ditches	[29]
akbD	Benzene, toluene, ortho-xylene, ethylbenzene, phenol	*Rhodococcus* sp. DK17 (AAR90134)	Oil-contaminated site	[30]
xylM	Toluene	*P. putida* mt-2 (CAC86827)	Field soil	[31]
t*odE*	Toluene	*P. putida* F1 (AAA26010)	Soil	[32-33]
tmoA	Toluene	*Pseudomonas mendocina* KR1 (AAA25999)	Algal-bacterial mat	[34]
tbuC	Toluene	*Ralstonia pickettii* PKO1 (AAB09623)	Benzene-, toluene-, ethylbenzene- and xylene(s)-contaminated aquifer	[35]
dmpL	Phenol	*P. putida* CF600 (AAA25940)	Unknown	[36-37]
bnzA1	Benzene	*Rhodococcus opacus* B4 (BAD95523)	Gasoline-contaminated soil	[38]
nahAc	Naphthalene	*P. putida* G7 (BAE92156)	Soil	[39-40]
narAa	Naphthalene	*Rhodococcus* sp. NCIMB12038 (AAD28100)	Garden soil with carbaryl	[41-42]
ndoB	Naphthalene	*P. putida* NCIB9816 (P0A110)	Garden soil	[43]
doxB	Naphthalene	*Pseudomona*s sp. C18 (P0A111)	Soil	[44]
nagAc	Naphthalene	*Ralstonia* sp. U2 (AAD12610)	Oil-contaminated soil	[45-46]
pahC	Naphthalene, phenanthrene	*P. putida* OUS82 (BAA20395)	PAH-contaminated soil	[47]
phnAc	Naphthalene, phenanthrene, anthracene	*Burkholderia* sp. RP007 (AAD09872)	PAH-contaminated site	[48]
phdA	Naphthalene, phenanthrene	*Nocardioides* sp. KP7 (BAA84712)	Ocean	[49]
nidA	Toluene, meta-xylene, naphthalene, benzo[a]anthracene, benzo[a]pyrene	*Mycobacterium vanbaalenii* PYR-1 (AAT51751)	Oil-contaminated sediment	[50-51]
C23O	Catechol	*P. putida* ND6 (AAP44220)	Industrial wastewater	[52]
catA/C12O	Catechol	*P. putida* ND6 (AAP44248)	Industrial wastewater	[52]
benA	Benzoate	*Halomonas organivorans* (CBR26855)	Saline environments	[53]
bssA	Benzene, toluene, ethylbenzene, xylene	*Thauera aromatica* K172 (CAA05052)	Activated sludge	[54]
nmsA	Naphthalene	*Deltaproteobacteria* sp. NaphS2 (CAO72219)	Marine sediment	[55]
bclA	Benzoyl-CoA	*Magnetospirillum* sp. LM-5 (CAA7611436)	Activated sludge	[56]
badA	Benzoyl-CoA	*Rhodopseudomonas palustris* (CAJ18317)	Waste water	[56]
bzdA	Benzoyl-CoA	*Azoarcus* sp. CIB (AAQ08820)	Diesel fuel-contaminated aquifer	[56]
bamY	Benzoyl-CoA	*Geobacter metallireducen* GS-15 (ABB32372)	Freshwater sediment	[56]

比如 *Rhodococcus erythropolis* 能在石蜡表面形成厚厚的生物被膜,同时伴随着细胞电势降低和细胞膜脂肪酸组分变化[63]；另一种是产生表面活性剂使石蜡乳化[65]。固体长链烷烃的降解酶有烷烃羟化酶和细胞色素 P450s，这两种酶的降解方式都为末端氧化。*Acinetobacter baylyi* ADP1 能降解 C_{12}−C_{36} 烷烃，其烷烃羟化酶为 AlkM[25]，是 AlkB 的同源蛋白。*Acinetobacter* sp. M-1 中有 2 个 *alkM* 的同源基因,命名为 *alkMa* 和 *alkMb*,催化 C_{20}−C_{44} 烷烃降解[66]。细胞色素 P450s 家族中 CYP116B5 酶使 *Acinetobacter radioresistens* 菌株能够以 C_{14}−C_{36} 为唯一碳源生长[67]。此外，降解固体长链烷烃还有 2 个代表性基因，分别为 *almA* 和 *ladA*。*Acinetobacter* sp. DSM 17874 菌株产生的 *almA* 是首个克隆出来的能够特异性降解碳链超过 C_{30} 的基因[23]，随后在其他菌属中都发现了 *almA* 的同源基因，但是该基因的降解机制尚不明晰。嗜热酶 LadA 是通过基因组学和蛋白质组学技术在 *Geobacillus thermodenitrificans* NG80-2 菌株中被发现，能够对 C_{15}−C_{36} 烷烃进行末端氧化[24]。

环烃类化合物通常很难降解，以环己烷为例，只有少数微生物能以环己烷为唯一碳源进行生长[68]。环己烷降解需要两种氧化酶的作用，一种氧化酶将环己烷氧化为环己醇，脱氢形成环己酮；另一种氧化酶则氧化环己酮为己内酯后开环继续降解，最终生成 CO_2 和 H_2O[11]。研究表明，环己烷单加氧酶为细胞色素 P450 蛋白家族，如 *Bacillus megaterium* 菌株利用细胞色素 P450BM3 (CYP102A1)氧化环己烷，菌株 *Acidovorax* sp. CHX100 利用细胞色素 P450 单加氧酶(CYP450chx)将环己烷氧化为环己醇[68-69]。

1.2 芳香烃的好氧微生物降解

芳香烃因含有苯环结构，相比于烷烃较难降解。在芳香烃的好氧降解过程中，芳环结构在加氧酶的作用下生成中心中间产物，主要包括邻苯二酚、原儿茶酸、龙胆酸盐和尿黑酸等，而后开环进一步降解，产物最终进入三羧酸循环[2,11,70-71]。

图 1 以甲苯降解为例来阐述苯系物(苯、甲苯、乙苯、二甲苯)好氧降解途径中所需的酶。*P. putida* mt-2 菌株是研究甲苯降解的模式菌株，其质粒 pWW0 上有 2 个 *xyl* 操纵子，分别编码甲苯降解上游途径和下游途径的酶。上游途径是将甲苯转化为苯甲酸，由操纵子 *xylUWCMABN* 完成；下游途径是将苯甲酸转化为三羧酸循环的中间产物，由操纵子 *xylXYZLTEGFJQKIH* 完成[72-73]。单加氧酶 XylMA 催化甲苯降解的初始反应，将甲基基团羟基化[35]。XylE 为邻苯二酚 2,3-双加氧酶(C23O)，可将邻苯二酚间位开环；该酶在不动杆菌属、考克氏菌属和假单胞菌属中均有发现[74]。XylR 和 XylS 则是转录正调控因子，分别激活上游途径和下游途径[35]。值得关注的是,Kim 等从土壤中分离出的 *Rhodococcus* sp. DK17 菌株具有两条不同的芳烃开环途径，能降解多种单环芳烃，如苯、甲苯、乙苯、邻二甲苯、苯酚等，其降解基因簇是 *akbBCDEF*，*akbS* 和 *akbT* 作为双组分调控系统调控这一基因簇的表达[30,75-76]。

作为最简单的多环芳烃，萘已经成为研究芳香烃代谢的模式物质。研究显示，萘降解途径也分为上游途径和下游途径。上游途径是将萘转化为水杨酸的过程，由操纵子 *nahAaAbAcAdBFCED* 完成，其中 NahAaAbAcAd 为双加氧酶，将萘转化为顺-1,2-二氢-1,2-萘二醇。下游途径中，操纵子 *sal* 或 *gen*/*sgp* 分别将水杨酸转化为邻苯二酚或龙胆酸[71]。邻苯二酚由操纵子 *nahGTHINLOMKJ* 通过间位开环途径最终转化为丙酮酸和乙醛；龙胆酸由操纵子 *nagGHAaAbILK* 转化为丙酮酸和富马酸[47,77-78]。萘的降解途径在不同菌株中呈现多样性，可能是降解基因发生基因水平转移所致[47,71]。以部分假单胞菌中 *nah* 样基因为例，*P. putida* NCIB9816 菌株 *ndo* 基因与 *nah* 基因功能相同[40,79]；*Pseudomonas* sp. C18 菌株 *dox* 基因的功能是氧化二苯并噻吩[44]；*P. putida* OUS82 和 *P. aeruginosa* PaK1 菌株中 *pah*

基因参与多环芳烃的降解[80-82]，上述菌株都可利用萘作为底物生长。尽管不同菌株的萘代谢途径各有不同，但是萘降解调控基因在不同菌株中高度保守,可作为功能基因标签[83]。萘双加氧酶基因 *nahAc* 是表征萘好氧降解的基因标签；同样地，其他芳香烃的双加氧酶基因也能作为功能基因标签[84]，如吡喃双加氧酶基因 *nidA*[50-51,85]、邻苯二酚 2,3-双加氧酶基因 C23O[86-88]和邻苯二酚 1,2-双加氧酶基因 *catA* (或 C12O 基因)[89]。此外，还有一些在细菌中普遍存在的芳环羟基化双加氧酶基因可作为功能基因标签的参考，如 *nag*、*nar*、*phn* 和 *bph*[90-91]，以及苯酚羟基化单加氧酶基因 *phe*[92]。

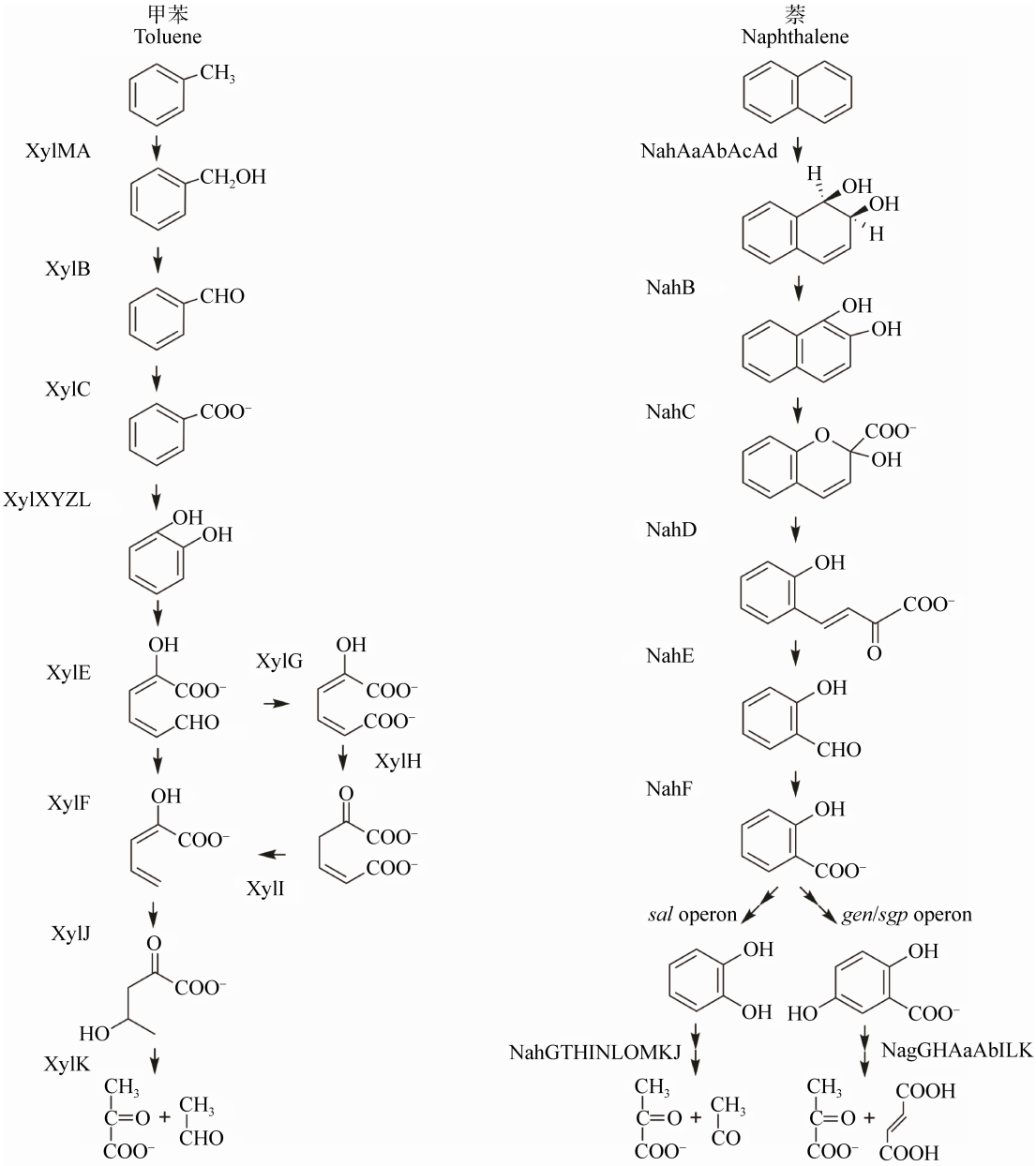

图 1　甲苯和萘的好氧降解途径

Figure 1　Aerobic degradation pathways of methylbenzene and naphthalene

2 微生物厌氧降解石油烃的关键酶与功能基因

2.1 烷烃的厌氧微生物降解

相较于好氧降解研究，厌氧降解的酶系统研究较少。延胡索酸加成是厌氧降解的主要途径之一，由甘氨酰基自由基酶催化，如烷基琥珀酸合酶(alkylsuccinate synthase，ASS)或甲烷基琥珀酸合酶(methylalkylsuccinate synthase，MAS)[58]。硫酸盐还原菌 *Desulfatibacillum alkenivorans* AK-01 和脱氮菌 *Aromatoleum* HxN1 分别是研究 ASS 和 MAS 的模式菌株[28-29]。硫酸盐还原菌 *D. alkenivorans* AK-01 能在 C_{13}–C_{18} 烷烃、十五碳烯和十六碳烯中生长，其基因组中包含 2 个不同的 *ass* 操纵子，参与底物激活、辅酶 A 连接、碳链重排和脱羧反应等烷烃代谢过程[29,93-94]。ASS/MAS 酶通常参与短链和中链烷烃的延胡索酸加成反应[95-96]，有研究显示 ASS 也能介导固态石蜡的延胡索酸加成：Wawrik 等[97]通过基因组学分析结合 *assA* 转录分析，发现海洋沉积物中的微生物能将固体石蜡 C_{25}–C_{50} 转化为甲烷。*assA* 和 *masD* 基因分别是 ASS 酶和 MAS 酶的主要编码基因，已成为鉴定厌氧烷烃降解微生物的基因标签[29,98]。

2.2 芳香烃的厌氧微生物降解

芳香烃类厌氧降解是在合酶、羧酶和脱氢酶的作用下生成中心中间产物苯甲酰辅酶 A 及其衍生物(如间苯二酚、间苯三酚、羟氢醌)，而后在还原酶作用下进一步降解为乙酰辅酶 A[70,99-100]。通常认为，无取代基芳香烃降解途径的初始反应由羧化作用激活，甲基化芳香烃降解途径的初始反应则由延胡索酸加成反应激活[100] (图 2)。

相较于甲基化芳香烃，无取代基芳香烃的降解研究较少。Meckenstock 等总结了无取代基芳香烃降解微生物、初始羧化反应及可能参与降解的酶[101]。Zhang 等通过稳定同位素标记分析出严格厌氧条件下萘降解的主要代谢产物萘甲酸，证实了萘降解途径的第一步反应是羧化作用[102]。因打开苯环中的 C–H 键是一个高耗能的过程，因此，苯的直接羧化作用研究在基因组学研究出现后才逐渐清晰。Abu Laban 等[103]通过聚丙烯酰胺凝胶电泳发现可能参与苯羧化作用的厌氧苯羧化酶 AbcA，Luo 等[104]通过转录组学分析也发现了类似基因。此外，苯厌氧降解的初始反应也能通过羟化作用实现。Zhang 等[105]发现 *Geobacter metallireducens* 在厌氧条件下将苯转化为苯酚后再进行下一步降解。氨基酸序列比对发现催化萘和苯的羧化酶属于 UbiD 酶家族[100]。尽管参与菲厌氧降解的酶尚属未知，但由于菲的主要代谢产物为菲甲酸，因而菲厌氧降解途径的初始反应被认为也是羧化作用[102,106]。目前，关于无取代基芳香烃羧化作用的证据几乎都是来自代谢产物分析和基因分析，具体酶催化的分子机制尚不明晰。

与烷烃厌氧降解的延胡索酸加成反应相似，甲基苯和甲基萘的延胡索酸加成途径需要琥珀酸苄基合酶(benzylsuccinate synthase，BSS)和萘甲基琥珀酸合酶(naphthylmethylsuccinate synthase，NMS)的参与[107] (图 2)。BSS 和 NMS 的 α 亚基编码基因分别为 *bssA* 和 *nmsA*[108]，已被用作基因标签来鉴定芳香烃厌氧降解微生物的存在[107,109]。苯系物经由 BssA 参与的延胡索酸加成反应，转化为核心中间产物苯甲酰 CoA，而后再由两类酶催化苯甲酰 CoA 实现初始去芳化[20,110]。一类酶为依赖于 ATP 的苯甲酰 CoA 还原酶 BcrCBAD/BzdNOPQ/BadDEFG，分布于 *Thauera aromatica*、*Azoarcus* spp.、*Aromatoleum* spp. 和 *Rhodopseudomonas palustris* 等兼性厌氧菌中；另一类为不依赖 ATP 的苯甲酰 CoA 还原酶 BamBCDEFGHI，分布于严格厌氧菌中，如 *Geobacter metallireducen*[107]。去芳化后的产物由水解酶 BamA/BzdY/Oah 逐步氧化，最终生成乙酰 CoA 和 CO_2[56,107]。与此类似，甲基萘经由 NmsA 参与的延胡索酸加成反应，生成中心中间产物萘甲酰 CoA，而后由萘甲酰 CoA 还原酶 Ncr 催化其初始的去芳化(图 2)。尽管萘甲

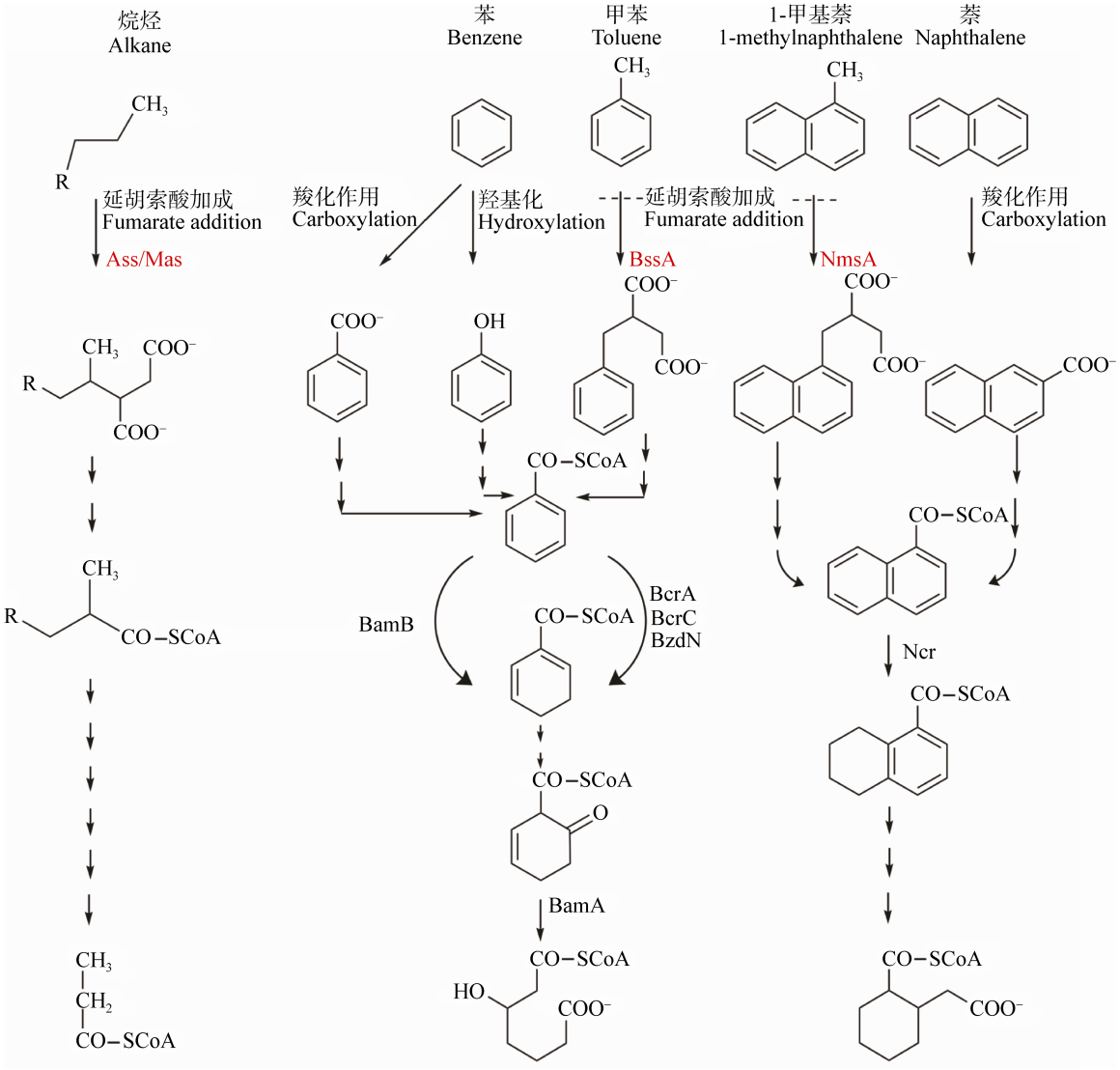

图 2　烷烃、苯、甲苯、萘和 1-甲基萘的厌氧降解途径

Figure 2　Anaerobic degradation pathways of alkane, benzene, toluene, naphthalene and 1-methylnaphthalene

酰 CoA 还原酶(Ncr)在一些硫酸盐还原菌中保守存在[111]，但仍需要提供其他类型的降解条件(如铁还原)或更多降解物(如菲)来共同确定 *ncr* 是否能作为基因标签[100]。

3　结论与展望

自然界中的微生物参与营养物质的循环和有机物的降解，与动植物产生互利共生关系，在维持生态系统平衡中发挥了重要作用。石油烃污染区域的微生物群落特征通常与未污染区域不同，通过深入研究污染物类型、环境条件和微生物群落等的各自特征与相互作用，有利于快速精准地实施高效的污染物微生物修复。随着高通量测序技术的快速发展，该技术在微生物降解与环境修复领域中得到了广泛应用，已成为石油降解微生物研究的重要手段。综合利用多种组学技术，挖掘与鉴定核心功能基因、解析关键代谢途径已成为研究降解基因多样性、自然衰减潜力与改造和构建基因工程菌等研究的重要基础。然而，由于功能基因的发现是建立在酶学研究的基础上，对石油烃的微生物代谢途径与

关键限速酶的解析与鉴定是发现新功能基因的主要限制因素。尽管一些石油烃降解功能基因被发现，但由于石油烃物质的多样性与复杂性，仍然难以满足科学研究与实际应用的需要。如由于复杂多环芳烃的微生物代谢途径尚不清楚，其关键酶的鉴定仍很匮乏，功能基因的研究鲜有报道，在一定程度上限制了此类污染物的微生物降解研究与环境修复进程。因此，后续研究应注重相关降解限速酶的功能研究与新代谢途径的明晰，进一步丰富和完善功能基因的数据库。

当利用功能基因研究微生物群落结构和功能时，在微生物降解底物的多样性和功能基因的特异性扩增方面需要关注。一种石油烃污染物通常能被多种微生物降解，一种微生物也可以同时降解多种石油烃污染物。Rojo[22]将石油烃降解微生物分为两类：一类为专一降解微生物，另一类为广谱降解微生物，后者能够同时催化降解饱和脂肪烃和芳香烃。之前的研究大多集中于单个降解微生物的分离鉴定和对单一石油烃类(如低分子量的 PAHs 和烷烃)的降解特征研究，对广谱性降解微生物的分类研究很少。随着高通量测序技术的快速发展，基因组与转录组相结合的功能基因鉴定方法已成为研究广谱石油烃降解菌的重要技术手段。Whyte 等[112]发现 *Pseudomonas* sp. BI7 含有烷烃和芳香烃降解基因，能同时降解烷烃和芳香烃，首次证明了广谱降解微生物的存在。此后，关于广谱降解微生物的研究报道逐渐增多，包括分枝杆菌属(*Mycobacterium*)、红球菌属(*Rhodococcus*)、假单胞菌属(*Pseudomonas*)、变形杆菌属(*Firmicutes*)等微生物，其中分枝杆菌属和红球菌属在混合石油烃降解中最常报道。*Mycobacterium vanbaalenii* PYR-1 菌株中单加氧酶 NidAB 和双加氧酶 NidA3B3 参与多种底物的代谢，不仅包括甲苯、间二甲苯、萘、苯并[a]蒽和苯并[a]芘，还包括咔唑和二苯并噻吩这些非烃类物质[113]。菌株 *P. aeruginosa* SJTD-1 利用 2 个 AlkB 单加氧酶、2 个 P450 单加氧酶和 1 个 AlmA 单加氧酶降解 C_{12}–C_{24} 烷烃[114-115]。利用多种底物进行生长是微生物进化的趋势，因此，在基于功能基因研究微生物降解底物时应重点考虑广谱降解菌的存在与特征[116]。

针对功能基因的特异性扩增和基因定量，在石油烃降解微生物的研究中有着重要的应用价值和科研意义。由于不同生物分类的功能基因其序列差异较大，因此往往需要设计简并引物对环境样品的功能基因进行扩增。Kloos 等[117]根据烷烃降解功能基因 *alkB* 的全长序列，与 GenBank 中已知的烷烃降解微生物的序列进行比对，设计了能够检测大多数 *alkB* 基因的简并引物，并对土壤样品 DNA 进行扩增和测序。Lueders 等[118]利用延胡索酸加成功能基因(*bssA*、*assA*、*bamA*、*bcrA* 等)的简并引物，通过 PCR 扩增、末端限制性片段长度多态性(T-RFLP)分析和扩增子测序，建立了一套直接快速检测石油烃厌氧降解固有菌群和降解潜力的方法。Shahsavari 等[84]则利用简并引物，通过实时荧光定量 PCR (qPCR)对石油烃污染土壤中降解多环芳烃的功能基因进行定量。需要注意的是，由于石油污染物成分复杂而降解基因多样性高，直接借鉴已有的简并引物可能会导致微生物群落功能研究有所疏漏。因此，可通过设计代谢通路中多个关键基因的简并引物，实现全面研究不同环境样品的功能基因多样性。

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