·综 述·

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# 细菌 Rieske 非血红素铁环羟化酶在多环芳烃降解中的研究进展

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**摘 要:** 多环芳烃是一种常见的持久性有机污染物，因具有致癌、致突变等毒性而被广泛关注。其微生物降解过程通常由羟化起始，随后脱氢、开环、一步步去除支链，最终进入三羧酸循环。Rieske 非血红素铁环羟化酶 (Rieske-type non-heme iron aromatic ring-hydroxylating oxygenases，RHOs，又称 aromatic ring-hydroxylating dioxygenases) 或细胞色素 P450 氧化酶负责将羟基加成到多环芳烃环上，将疏水性的多环芳烃转化为亲水性的衍生物，这一过程是多环芳烃降解转化的起始步骤，也是关键步骤和限速步骤之一。文中主要介绍 RHOs 的分布、底物特异性、底物识别机制以及研究 RHOs 与多环芳烃的一些技术和方法等，并对 RHOs 在环境修复技术中的潜在应用进行了展望。

**关键词:** Rieske 非血红素铁环羟化酶，多环芳烃，底物范围，底物识别，环境修复

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# Advances in bacterial Rieske non-heme iron ring-hydroxylating dioxygenases that initiate polycyclic aromatic hydrocarbons degradation

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**Abstract:** Polycyclic aromatic hydrocarbons (PAHs) are a class of persistent organic pollutants, which have received widespread attentions due to their carcinogenic and mutagenic toxicity. The microbial degradation of PAHs are usually started from the hydroxylation, followed by dehydrogenation, ring cleavage and step-by-step removal of branched chains, and finally mineralized by the tricarboxylic acid cycle. Rieske type non-heme iron aromatic ring-hydroxylating dioxygenases (RHOs) or cytochrome P450 oxidases are responsible for the conversion of hydrophobic PAHs into hydrophilic derivatives by the ring hydroxylation. The ring hydroxylation is the first step of PAHs degradation and also one of the rate-limiting steps. Here, we review the distribution, substrate specificity, and substrate recognition mechanisms of RHOs, along with some techniques and methods used for the research of RHOs and PAHs.

**Keywords:** Rieske non-heme iron ring-hydroxylation dioxygenases, polycyclic aromatic hydrocarbons (PAHs), substrate range, substrate recognition, environmental remediation

## 1 多环芳烃和 RHOs 双加氧酶

### 1.1 多环芳烃

多环芳烃 (Polycyclic aromatic hydrocarbons，PAHs) 是一种常见的持久性有机污染物，其分子结构中含有两个及以上苯环，疏水性强，水溶性低，能够稳定地存在于环境中，不易被降解[1]，因其具有致癌、致突变等毒性而被广泛关注[2]。多环芳烃根据分子量的高低，分为由萘、菲、蒽等 2 个或 3 个稠环组成的低分子量多环芳烃和由荧蒽、芘、苯并[a]蒽和苯并[a]芘等 4 个或 4 个以上环组成的高分子量多环芳烃[3]。在国内外，多环芳烃都是非常受关注的环境污染物。1995 年，美国环保署就将 16 种多环芳烃列为优先控制的污染物[4]，我国《土壤环境质量建设用地土壤污染风险管控标准 (试行)》(GB36600-2018)[5]等国家标准将萘、苯并[a]蒽等 8 种 PAHs 列入环境污染风险筛选和管控范围 (图 1)。目前，多环芳烃有物理、化学和生物等降解方式[6]。其中，生物降解是一种自然降解方式，占环境中多环芳烃降解的 40%–60%[7]，是去除或者降低环境中多环芳烃污染的一种重要方式[8]。当前已经从多环芳烃污染的环境中分离到多种以多环芳烃为唯一碳源的菌株，如能降解萘、菲的 *Diaphorobacter* sp.、寡养单胞菌属 *Stenotrophomonas* sp.[9-10]，以及能够降解高分子量多环芳烃荧蒽、芘的红球菌属 *Rhodococcus* sp.、假单胞菌属 *Pseudomonas* sp.、鞘氨醇盒菌属 *Sphingopyxis* sp.、芽孢杆菌属 *Bacillus* sp.[1,11]等。国内很多实验室也分离获得对萘[12]、菲、荧蒽[13]、苯并[a]芘[14]等多种多环芳烃具有高效降解作用的菌株，比如恶臭假单胞菌 *Pseudomonas putida* B6-2 对 13 种多环芳烃及 7 种二噁英都具有一定的降解能力[15]，而油菜假单胞菌 *Pseudomonas brassicacearum* MPDS 对多环芳烃和杂环衍生物都具有高效的降解能力[16]。另外，我国科学家在多环芳烃污染的来源、时间空间分布[17]、健康风险评估[18-19]、生物修复[20]、代谢途径 (如下游水杨酸途径[21]、龙胆酸代谢途径[22]等) 以及相关酶 (如环羟基化酶[23]等) 分子

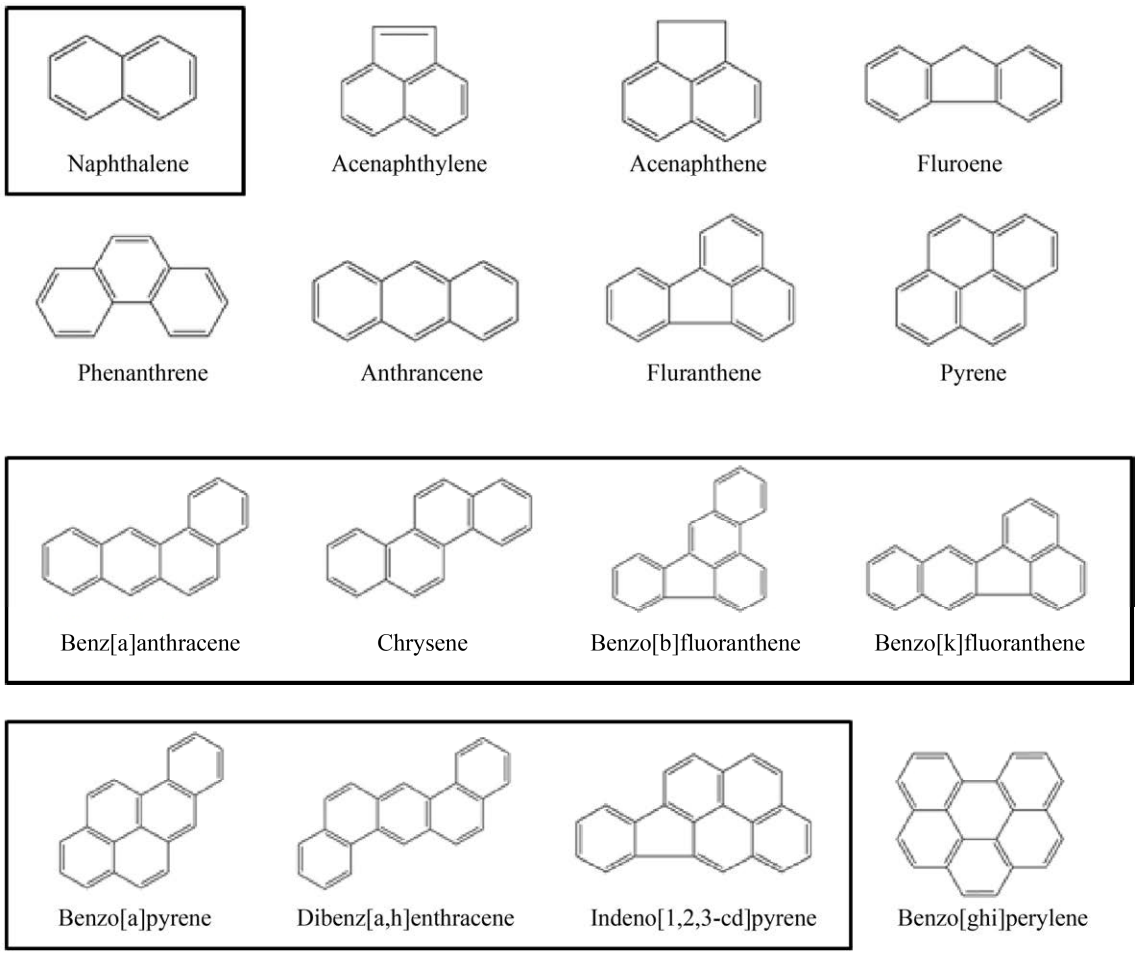


**图 1　美国环保署优先控制的 16 种多环芳烃 (黑色框为国内管控的 8 种多环芳烃)**
Fig. 1　Sixteen PAHs regulated by EPA of US (black boxed are the eight PAHs regulated in China).

机制研究等方面做了大量的突出工作。目前低分子量多环芳烃及其下游代谢途径的研究已经比较清楚，尤其是萘、菲、蒽、芴等的降解途径[24]。而由于高分子量多环芳烃的水溶性更差、代谢途径更长，也更复杂多样化[25]，对于细菌中高分子量多环芳烃降解途径的研究不甚透彻，在已经报道的高分子量多环芳烃降解菌株中的具体代谢途径及其中所涉及的酶的鉴定及其性质等还需进一步探究。可以预见在较长一段时间内，针对高致畸性高危害性高分子量多环芳烃的降解机制和代谢途径研究将是多环芳烃研究的重点。

### 1.2　多环芳烃降解关键酶——RHOs

多环芳烃的降解起始于羟化，然后脱氢、开环、一步步去除支链，最终进入三羧酸循环 (Tricarboxylic acid cycle，TCA cycle) 被微生物所利用。微生物降解多环芳烃的第一步反应即羟化反应，这一步在细菌中研究较多的酶是 Rieske 非血红素铁环羟基化酶 (Rieske-type non-heme iron aromatic ring-hydroxylating dioxygenases，RHOs)，在真菌中研究较多的是细胞色素 P450 单加氧酶 (Cytochrome P450 monooxygenase，CYP450)。RHOs 能催化多种氧化反应，包括碳-碳键的氧化裂解、单羟基化和二羟基化反应[26]。这种酶是唯

一已知的催化邻位顺式二醇立体选择性一步形成的酶[27]，能在一个酶促步骤中将两个羟基引入到多环芳烃上形成顺式二氢二醇 (图 2)[28]。顺式二氢二醇经过脱氢酶脱氢后形成相应的二醇，在内二醇或外二醇双加氧酶的作用下开环，内二醇双加氧酶作用于两个羟基之间的 C-C 键，而外二醇双加氧酶切割与两个羟基相邻的 C-C 键底物[29]。例如，PhdF 就是一种Ⅰ型外二醇双加氧酶，是邻位氧螯合超家族的成员，可作用于 3,4-二羟基菲等含有双羟基的苯环上，发挥开环的功能[30]。CYP450 在人类、植物、微生物中是广泛存在的，而且其底物范围非常广泛，能够催化 C-羟基化及杂原子氧合、释放和环氧化物的形成等[31]。CYP450 参与了 PAHs 生物降解过程的初始氧化步骤，催化多环芳烃的不同位置形成一种或多种单羟基化产物[32]，或者与环氧化物水解酶协同作用催化形成反式-二氢二醇 (图 3)[33-34]。CYP450 主要在真菌中研究的较多，如白腐真菌[35]、黄孢原毛平革菌，且能够作用于四环、五环乃至六环的苯并[ghi]苝等高分子量的多环芳烃[36]，细菌中少见报道。而在 *Rhodococcus* sp. P14[37]和 *Mycobacterium vanbaalenii* PYR-1[38]中都提到存在 RHOs 与 CYP450 共同降解多环芳烃的现象，这些菌能同时催化高分子量 PAHs 产生顺式-二氢二醇和反式-二氢二醇。而且研究者证明在多株 *Mycobacterium vanbaalenii* 菌中都有 RHOs 与 CYP450 基因共同存在的情况[38]。前面提到，内二醇以及外二醇双加氧酶只能识别两个羟基中间或者与两个羟基相邻的 C-C 键，而 CYP450 作用多环芳烃后一部分仅产生一个羟基形成酚，因而推测在 CYP450 羟化后，可能由 CYP450 再进行一次羟化，或者由 RHOs 对 CYP450 单羟基化

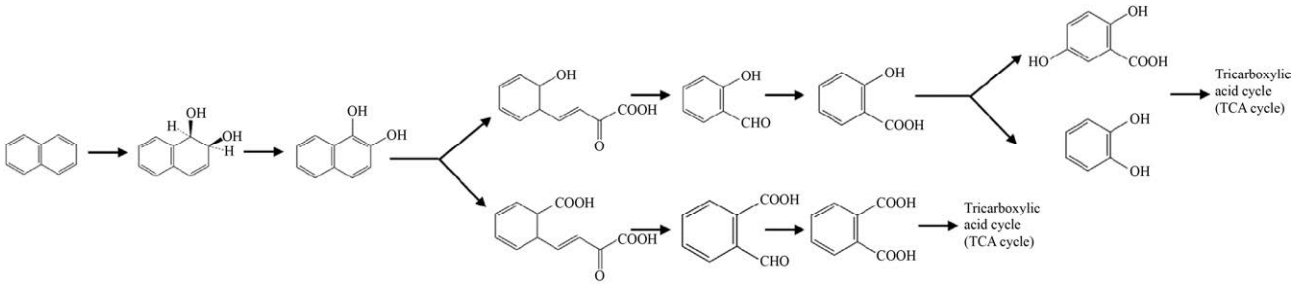


**图 2　RHOs 起始催化萘的降解途径**

Fig. 2　Degradation pathway of naphthalene initiated by RHOs.

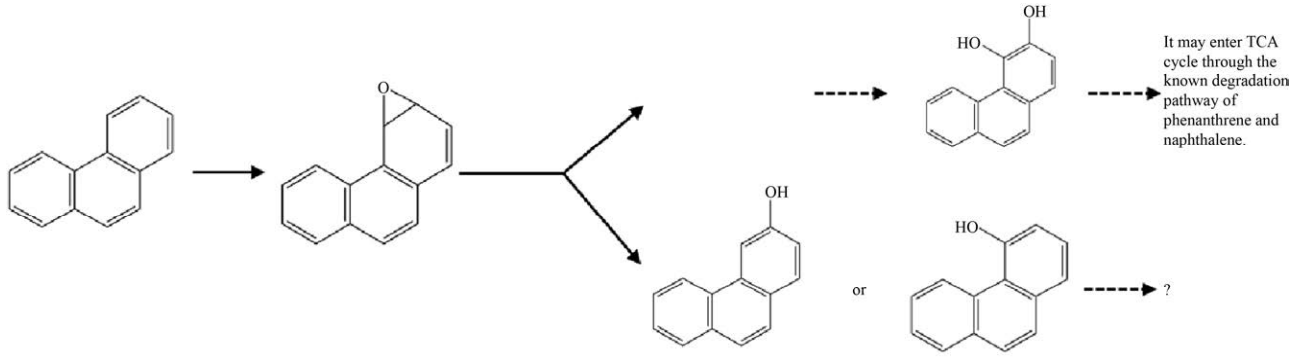


**图 3　CYP450 催化菲**

Fig. 3　Catalytic activity of CYP450 on phenanthrene.

的产物再次羟基化，最终形成开环酶能够作用的二醇的结构。而且早期也有文章提到 RHOs 能够催化单加氧反应[39]，如甲苯单加氧酶可能催化苯酚生成邻苯二酚[40-41]，萘双加氧酶也被证实能参与多种苄基单羟基化反应[42]。也有证据表明萘双加氧酶能够催化荧蒽和芴单羟基化分布形成 8-羟基荧蒽和 9-芴醇[43]，以及其他的研究中 RHOs 催化的单羟基化产物的出现[44]。RHOs 作为高分子量多环芳烃降解的两类起始酶之一，一旦确定特定多环芳烃可以被某一 RHOs 氧化后，即有可能根据基因组分布、代谢物分析等推测相关代谢中间物和中间途径，具有“提纲挈领”的意义。但是 RHOs 的生化研究过程中存在诸多需要被克服的困难。从技术上来说，首先，多环芳烃特别是高分子量多环芳烃溶解性差[24]，在体外酶活系统构建中与酶的结合能力差，需要有机溶剂溶解，但有机溶剂又可能对酶活性造成损伤[43]。RHOs 体外酶活系统中所需的电子传递体中的铁氧还蛋白还原酶 (Ferredoxin reductase，FdR) 难以纯化表达[43]，给体外酶活测定带来一定的困难，选择外源的电子传递体不能保证双加氧酶的酶活效率，在确定合适的电子传递体前，酶的活性难以用传统的 NADH 的消耗来表征；另外，由于高分子量的多环芳烃环数多，RHOs 作用的位置难以确定，产生一种甚至多种产物[45]，而对于产物的鉴定多采用的是气相色谱-质谱联用技术 (GC-MS)，因为产物缺少标准品或者标准品价格昂贵，使得研究方法受限。而在生信分析中，RHOs 的底物范围非常广泛，以萘双加氧酶 (Naphthalene dioxygenase，NDO) 为例[42]，它可以以多种物质为底物，这也为酶的底物确定带来了一定的困难。

综上，细菌 RHOs 在多环芳烃的降解途径中发挥着非常重要的作用，也存在着一定的研究难度和迫切之处，下面我们将对与多环芳烃降解相关的 RHOs 进行详细的论述。

## 2 Rieske 非血红素铁环羟基化双加氧酶 (RHOs) 的来源、组成

### 2.1 RHOs 的菌株来源分布

目前对于 RHOs 的研究集中在 *Rhodococcus* sp.、*Pseudomonas* sp.、罗尔斯通氏菌属 *Ralstonia* sp.、海杆菌属 *Marinobacter* sp.、丛毛单胞菌属 *Comamonas* sp.、潘多拉菌属 *Pandoraea* sp.等菌株中[46]。表 1 统计了 1988 年至今能够使用如 Naphthalene dioxygenase、Biphenyl dioxygenases 等特定关键词查询到的在文献中发表的已经与多环芳烃降解相关的 RHOs 基因，少有发现新型的 RHOs，进行表征的都与早期发现的 RHOs 具有高度同源性。在多环芳烃降解菌的筛选及研究过程中还发现 RHOs 可能在不同菌属中存在水平转移的现象。早期有观点认为 RHOs 可能起源于革兰氏阴性细菌，随后转移到革兰氏阳性细菌中[47]。水平转移的现象被研究者们多次提到，如：利用简并 PCR 引物从 *Marinobacter* 中扩增萘双加氧酶的大亚基，经系统发育树分析，该萘双加氧酶与 *Pseudomonas* 和伯克氏菌属 *Burkholderia* 来源的萘双加氧酶相似，而在其基因簇附近发现了 *tnpA1* 基因的同源基因，可能编码噬菌体 λ 型转座酶，这可能解释了多环芳烃降解基因在这些细菌谱系中发生的水平转移[48]。同样的，以来自 *Pseudomonas* spp.的 2Fe-2S 还原酶基因设计引物，发现食酸菌属 *Acidovorax* 中有与 *Pseudomonas* spp.相同的萘双加氧酶的相关基因，但是属于同一物种的菌株则没有[49]。研究推测这种基因水平转移的发生在微生物群落适应污染物中发挥了重要作用[50]。

### 2.2 Rieske 非血红素铁环羟基化酶 (RHOs) 的组成

Rieske 非血红素铁环羟基化酶 (RHOs) 是一种多组分酶系统，由末端双加氧酶、铁氧还蛋白 (Ferredoxin，Fd)、铁氧还蛋白还原酶 (Ferredoxin

**表 1 RHOs 来源及其作用的底物**

**Table 1 Origins and substrates of RHOs**

| GenBank | Strains | RHOs | Substrates | Year | References |
|---|---|---|---|---|---|
| CP054128 | *Pseudomonas* sp. MPDS | Naphthalene dioxygenase | Naphthalene, fluorene, dibenzofuran and dibenzothiophene | 2021 | [16] |
| MH560349 | *Pseudomonas fluorescens* AH-40 | Naphthalene dioxygenase | Phenanthrene | 2020 | [51] |
| KJ461700 | *Pseudomonas aeruginosa* N6P6 | Naphthalene dioxygenase | Naphthalene | 2020 | [52] |
| MULN00000000 | *Pseudomonas veronii* strainVI4T1 | Naphthalene dioxygenase | Naphthalene | 2019 | [53] |
| JN613334 | *Rhodococcus wratislaviensis* strain 9 | Naphthalene dioxygenase | Phenanthrene | 2019 | [54] |
| AAD28100 | *Rhodococcus* sp. strain NCIMB12038 | Naphthalene dioxygenase | Naphthalene | 2019 | [55] |
| CP006254 | *Geobacillus* sp. JF8 | Naphthalene dioxygenase | Naphthalene | 2019 | [56] |
| NO | *Sphingobium yanoikuyae* B1 | Biphenyl 2,3-dioxygenase | Phenazine | 2017 | [57] |
| KC771235 | *Pseudomonas aeruginosa* JP-11 | Biphenyl dioxygenase | Biphenyl | 2016 | [58] |
| JN235141 | *Rhodococcus* sp. ustb-1 | Naphthalene dioxygenase | Pyrene | 2015 | [59] |
| Q46372 | *Pandoraea pnomenusa* B356 | Biphenyl dioxygenases | 3-hydroxy-4,4′-dichlorobiphenyl, 3,3′-dihydroxy-4,4′-chlorobiphenyl, flavone, isoflavone, and flavanone | 2015 | [60] |
| KM102520 | Uncultured *gammaproteobacterium* | Aromatic ring-hydroxylating oxygenases (phd20/19) | Biphenyl, naphthalene, phenanthrene, pyrene, fluoranthene | 2014 | [61] |
| KM102522 | Uncultured *gammaproteobacterium* | Aromatic ring-hydroxylating oxygenases (bph29/28) | Biphenyl, naphthalene | 2014 | [61] |
| KM102523 | Uncultured *gammaproteobacterium* | Aromatic ring-hydroxylating oxygenases (nah33/32) | Biphenyl, naphthalene, phenanthrene | 2014 | [61] |
| JN655512 | *Comamonas* sp. MQ | Naphthalene dioxygenase | Indole and most indole derivatives | 2013 | [62] |
| NO | *Martelella* sp. AD-3 | Naphthalene dioxygenase | Anthracene | 2012 | [63] |
| P37333 | *Pseudomonas* strain LB400 | A variant biphenyl dioxygenase | Dibenzofuran | 2012 | [64] |
| GQ184726 | *Burkholderia* sp. C3 | *Nag*-like dioxygenases | Naphthalene, dibenzothiophene | 2011 | [65] |
| GQ184727 | *Burkholderia* sp. C3 | *Phn*-like dioxygenases | Naphthalene, phenanthrene, dibenzothiophene | 2011 | [65] |
| Q53122 | *Rhodococcus jostii* RHA1 | Biphenyl and ethylbenzene dioxygenases | Polybrominated diphenyl ethers | 2011 | [66] |

(待续)

(续表 1)

| GenBank | Strains | RHOs | Substrates | Year | References |
|---|---|---|---|---|---|
| SRA028415 | Sediment metagenome | Novel aromatic ring hydroxylating dioxygenases | Biphenyls | 2011 | [67] |
| DQ846881 | *Rhodococcus opacus* R7 | Naphthalene dioxygenase | Naphthalene | 2010 | [68] |
| Q3C1D5 | *Comamonas* sp. strain E6 | Terephthalate 1,2-dioxygenase | Terephthalate | 2008 | [69] |
| Q53122 | *Rhodococcus jostii* RHA1 | Biphenyl and ethylbenzene dioxygenases | Styrene and benzene | 2008 | [70] |
| Q53122 | *Rhodococcus jostii* RHA1 | Biphenyl 2,3-dioxygenase | Biphenyl/polychlorinated-biphenyl | 2007 | [71] |
| HE577117 | Uncultured bacterium | Biphenyl dioxygenases | Biphenyls | 2007 | [72] |
| EF152282 | *Sphingobium yanoikuyae* B1 | Biphenyl/naphthalene dioxygenase | Biphenyl, naphthalene, and phenanthrene, toluene, m- and p-xylene | 2007 | [73] |
| AF295032 | *Marinobacter* strain NCE312 | Naphthalene dioxygenase | Naphthalene and 2-methylnaphthalene | 2006 | [48] |
| Q46372 | *Pandoraea pnomenusa* B356 | Biphenyl dioxygenases | 2-hydroxy-3-chlorobiphenyl, 2-hydroxy-5-chlorobiphenyl and 2-hydroxy-3,5-dichlorobiphenyl | 2004 | [74] |
| P37333 | *Pseudomonas* strain LB400 | Biphenyl dioxygenases | 2-hydroxy-3-chlorobiphenyl, 2-hydroxy-5-chlorobiphenyl and 2-hydroxy-3,5-dichlorobiphenyl, 2,2′-dichlorobiphenyl | 2004 | [74-75] |
| O52382 | *Ralstonia* sp. strain U2 | Naphthalene dioxygenase | Naphthalene | 2002 | [76] |
| AF061751 | *Burkholderia* sp. strain RP007 | *Phn*-like dioxygenases | Naphthalene, phenanthrene | 1999 | [77] |
| Q46372 | *Comamonas testosteroni* strain B-356 | Biphenyl dioxygenases | Biphenyl/chlorobiphenyl dioxygenase | 1996 | [78] |
| A5W4F2 | *Pseudomonas putida* F1 | Toluene dioxygenase | Benzene and toluene | 1994 | [79] |
| Q52438 | *Pseudomonas* sp. strain KKS102 | Biphenyl 2,3-dioxygenase | Biphenyl and polychlorinated biphenyls | 1994 | [55] |
| Q07944 | *Pseudomonas putida* ML2 | Benzene dioxygenase | Benzene | 1993 | [80] |
| Q52028 | *Pseudomonas pseudoalcaligenes* KF707 | Biphenyl dioxygenase | Biphenyls and polychlorinated biphenyls | 1992 | [81] |
| P37333 | *Pseudomonas* strain LB400 | Biphenyl dioxygenases | Polychlorinated-biphenyl | 1992 | [82] |
| P0A110 | *Pseudomonas putida* strain NCIB9816 | Naphthalene dioxygenase | Indole | 1988 | [83] |

reductases，FdR)[84]三部分组成 (图 4)。末端加氧酶一般含有大亚基和小亚基，构成 $\alpha_3\beta_3$ 异六聚体结构，如 *Sphingobium yanoikuyae* B1 中的联苯双加氧酶[85]；或者是只含有大亚基，构成 $\alpha_3$ 同三聚体，如咔唑的末端加氧酶成分 (PDB ID：4NBD)[86]。末端双加氧酶的 α 亚基包含一个 Rieske [2Fe-2S]簇和一个单核铁活性中心。Fd 含有一个 Rieske 型的[2Fe-2S]簇[87]，而 FdR 是由 3 个结构域组成：FAD 结合结构域、NADH 结合结构域和 C 末端结构域[88]，有些菌中的 Fd 和 FdR 可能融合形成一个蛋白，如食醚红球菌 *Rhodococcus aetherivorans* IcdP1[89] (GenBank 登录号：CP011341) 中的 RHOs 加氧酶组分附近分布着一些潜在的天然融合 NAD/FAD 或 FMN/NAD 结合域和[2Fe-2S]簇的蛋白，可能是为 RHOs 的加氧酶组分发挥电子传递作用，这种融合蛋白发挥电子传递作用早在 1992 年就有报道，如黄素蛋白与[2Fe-2S]簇的天然融合[90]。RHOs 的 3 个组分行使功能时，首先是 FdR 从 NAD(P)H 中释放电子，并将电子转移到 Fd 中，Fd 将电子转移到加氧酶[91]，加氧酶的 Rieske [2Fe-2S]簇从电子转移组分接受电子后，将电子转移到活性位点的单核铁，激活分子氧，从而攻击底物[26]。

## 3 RHOs 双加氧酶的底物范围

萘双加氧酶 (Naphthalene dioxygenase，NDO) 是与多环芳烃降解相关的 Rieske 非血红素铁环羟化酶中研究较多的一类。萘双加氧酶有多种类型，*nag* 类基因是从 *Pseudomonas putida* strain G7 首次发现的能够降解萘的基因，*phn* 是从 *Burkholderia* sp. RP007 中发现的能以萘、菲和蒽为唯一碳源的萘降解基因[65]。NDO 的底物范围非常广，可作用于萘之外的多种物质，具体可参考表 1 所统计的部分 RHOs 的底物。例如，荧光假单胞菌 *Pseudomonas fluorescens* AH-4、*Rhodococcus wratislaviensis* strain 9 的 NDO 能氧化菲[51,54]。萘双加氧酶还能作用于吲哚产生靛蓝或靛玉红[92]；鞘氨醇单胞菌 *Sphingomonas* CHY-1 中的 NDO 能羟化荧蒽、苯并[a]蒽、䓛、苯并[a]芘、芘，其中，

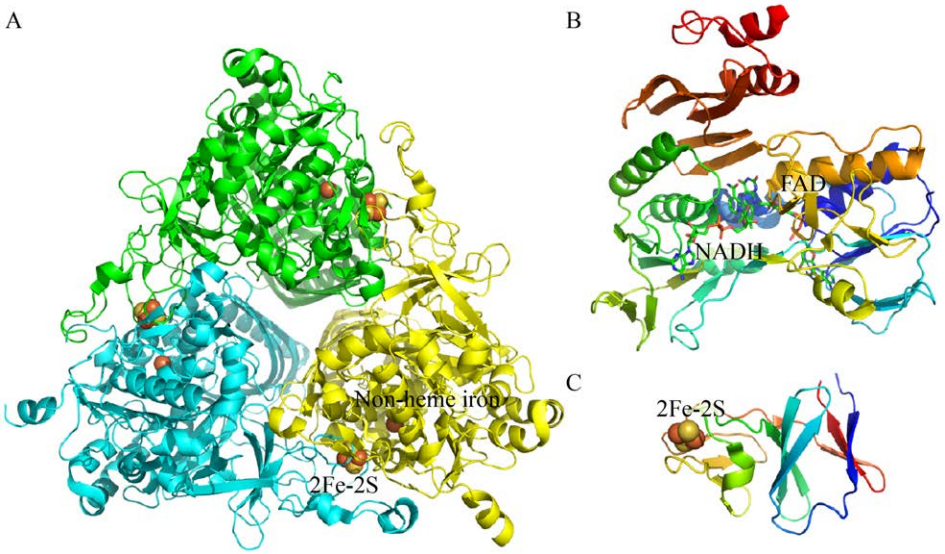


**图 4　RHOs 的各组成部分的代表性结构**

Fig. 4　Structures of three RHOs components. (A) Dioxygenase component (PDB ID: 2CKF). (B) Ferredoxin reductase (PDB ID: 1F3P). (C) Ferredoxin (PDB ID: 2QPZ).

苯并[a]芘的分子量虽然比芘更大，但是 NDO 作用于苯并[a]芘时的酶活更高，可能是在与 NDO 接触时，苯并[a]芘多出来的一个环更能靠近狭窄的酶活性中心[93]。另外 RHOs 中还有一类与联苯型多环芳烃相关的联苯双加氧酶 (Biphenyl dioxygenase, BPDOs) 的研究也相对较多。BPDOs 作用的底物多与多氯联苯及其衍生物相关，如 *Pandoraea pnomenusa* B356 的 BPDOs 能够催化联苯，后续发现它可将 5-氯-2-羟基联苯转化为 5-氯-2-羟基苯甲酸酯，还可参与对双对位氯取代联苯类似物 (3-羟基-4,4′-二氯联苯和 3,3′-二羟基-4,4′-氯联苯) 的代谢[60]。*Pseudomonas* strain LB400 也可降解联苯，同 B-356 BPDOs 一样具有可以催化 2-羟基-3-氯联苯、2-羟基-5-氯联苯和 2-羟基-3,5-二氯联苯的能力[74]。另外 LB400 还具有催化 2,2′-二氯联苯的能力[75]。除此之外，有研究者还发现 *Rhodococcus jostii* RHA1 中的联苯和乙苯双加氧酶在联苯、乙苯等底物存在下能够转化多溴二苯醚 (PBDEs)[66]。

## 4 基于结构生物学的底物识别机制研究

RHOs 可以催化多种反应，与许多不同底物形成复合物的结构表明，活性位点中底物的取向不仅控制区域特异性和立体特异性，而且还控制催化的反应类型[94]。在 RHOs 结合底物后，没有发现明显的侧链重排[91]，底物与单核铁活性中心接近的两个碳被羟基化，这解释了由 RHOs 产生的顺式二氢二醇的极端区域和立体选择性[91]。底物必须与 RHOs 充分相互作用，以防止催化时底物发生运动，催化活性口袋内部若存在多余空间可能会影响 RHOs 在发生催化作用时底物在口袋内部的活动[95]。

表 2 所示是 PDB 数据库中部分已解析结构的 RHOs。RHOs 利用一个单核非血红素铁中心来进行催化，此过程消耗了 2 个电子、2 个质子和 1 个氧气分子，产生了顺式二氢二醇。NDO 的结构表明活性位点口袋内的氨基酸大部分是疏水性的，这为芳香族底物的结合提供了合适的环境[96]。*Pseudomonas* sp. NCIB 9816-4 的 NDO 结构是第一个被解析的 RHOs[97]。它的第 352 位突变影响了萘的立体选择性，206 位和 295 位影响了联苯和菲的区域选择性，在同时突变 206/352 或者 206/295/352 的情况下，NDO 催化菲形成菲顺式-9,10-二氢二醇作为主要产物[98]，表明催化位点附近的关键残基影响 RHOs 的底物识别。

相对于催化萘的 RHOs (图 5A) 来说，结合高分子量多环芳烃的 RHOs 的疏水性底物的结合口袋明显更大，如 *Sphingomonas* CHY-1 的 RHOs，CHY-1 的 RHOs 是比较典型的能够羟基化五环 PAH 的酶，它的催化腔的中心区域主要由 Phe350、Phe404 和 Leu356 的侧链形成，形成相当均匀的梯形腔，从而影响酶的位置特异性[99]。另外，天然和联苯结合形式的 BPDO-OB1 结构与萘 1,2-双加氧酶相似，但活性位点入口也明显大于萘 1,2-双加氧酶的入口，活性位点残基的差异也允许高分子量多环芳烃蒀和苯并[a]芘的结合[100]。除此之外，RHOs 活性位点入口存在的柔性环也被认为是一种可以扩大底物特异性范围的特征，这个环可以在需要时提供重塑活性位点所需的结构灵活性，有利于不同配体的调节[61]。CHY-1 的 RHOs 的柔性环 L1 上的 Leu223 和 L2 上的 Ile260 有助于催化位点对高分子量多环芳烃的选择性 (图 5B)。同样，在 $BphAE_{LB400}$ 的 α 亚基上的残基也有一段覆盖活性位点的环，在底物结合时，该片段发生了明显的位移[95]。在鞘氨醇菌属 *Sphingobium* sp. FB3 中 RHOs 的加氧酶组分，223 位是 Phe，比能羟基化五环 PAH 的 *Sphingomonas* CHY-1 的 RHOs 的 Leu 大得多，突变该位点后 FB3-RHOs 降解苯并[a]芘速率加快[14]。这也说明关键残基的改变就可能改变酶的

底物特异性，再如 BphAEs 从 280 到 283 位于催化腔的入口，而 283 位的 Ser 被 Met 取代后，改变了催化位点内底物的方向，从而改变了其羟基化位点，增强了它对于多种多氯联苯的特异性。也正说明 283 位的突变对 BPDO 的底物特异性有显著影响[101]；BPDO 中与底物识别和区域特异性相关的位点被突变后也改变了 BPDO 在苯环上的作用位点[102]。

**表 2　已解析结构的 RHOs**

**Table 2　RHOs with resolved structure**

| PDB ID | Strains | RHOs | Substrates | Year | References |
|---|---|---|---|---|---|
| 4HJL, 4HKV, 4HM0-8 | *Pseudomonas* sp. C18 | Naphthalene 1,2-dioxygenase | 1-chloronaphthalene, benzamide, indole-3-acetate, thioanisole, styrene, indene, phenetole, indan, ethylbenzene, ethylphenylsulfide, 1-indanone | 2012 | [103] |
| 2YFI | *ParaBurkholderia xenovorans* LB400 | Biphenyl dioxygenase variant Rr41 | Dibenzofuran | 2011 | [104] |
| 2XR8 | *ParaBurkholderia xenovorans* LB400 | Biphenyl dioxygenase | Many polychlorinated biphenyls | 2010 | [105] |
| 3EN1 | *Pseudomonas putida* | Toluene 2,3-dioxygenase | Toluene | 2010 | [88] |
| 3GZY | *Comamonas testosteroni* sp. strain B-356 | Biphenyl dioxygenase | Biphenyl and polychlorinated biphenyls | 2009 | [106] |
| 2CKF | *Sphingomonas* sp. CHY-1 | PAH-hydroxylating dioxygenase | Fluoranthene, benz[a]anthracene, benzo[a]pyrene | 2007 | [99] |
| 2HMM | *Pseudomonas* sp. | Naphthalene 1,2-dioxygenase | Anthracene | 2006 | [91] |
| 2HML | *Pseudomonas* sp. | Naphthalene 1,2-dioxygenase mutant | Phenanthrene | 2006 | [91] |
| 2HMK | *Pseudomonas* sp. | Naphthalene 1,2-dioxygenase | Phenanthrene | 2006 | [91] |
| 2GBX | *Sphingomonas yanoikuyae* B1 | Biphenyl 2,3-dioxygenase | Biphenyl | 2006 | [100] |
| 2DE7 | *Janthinobacterium* | Carbazole 1,9a-dioxygenase | Carbazole | 2006 | [107] |
| 2B24 | *Rhodococcus* sp. NCIMB 12038 | Naphthalene 1,2-dioxygenase | Indole | 2005 | [108] |
| 2BMR | *Comamonas* sp. JS765 | Nitrobenzene dioxygenase | 3-nitrotoluene | 2005 | [109] |
| 1WQL | *Pseudomonas fluorescens* Ip01 | Cumene dioxygenase | Cumene or toluene | 2004 | [110] |
| 1WW9 | *Janthinobacterium* sp. J3 | Carbazole 1,9a-dioxygenase | Carbazole | 2005 | [111] |
| 1UUV | *Pseudomonas putida* | Naphthalene 1,2-dioxygenase | Nitric oxide and indole | 2004 | [112] |
| 1ULI | *Rhodococcus jostii* RHA1 | Biphenyl dioxygenase | Biphenyl | 2003 | [113] |
| 1O7G | *Pseudomonas putida* | Naphthalene 1,2-dioxygenase | Naphthalene | 2002 | [114] |
| 1EG9 | *Pseudomonas putida* | Naphthalene 1,2-dioxygenase | Indole | 2000 | [115] |
| 1NDO | *Pseudomonas putida* | Napthalene 1,2-dioxygenase | Naphthalene | 1998 | [116] |

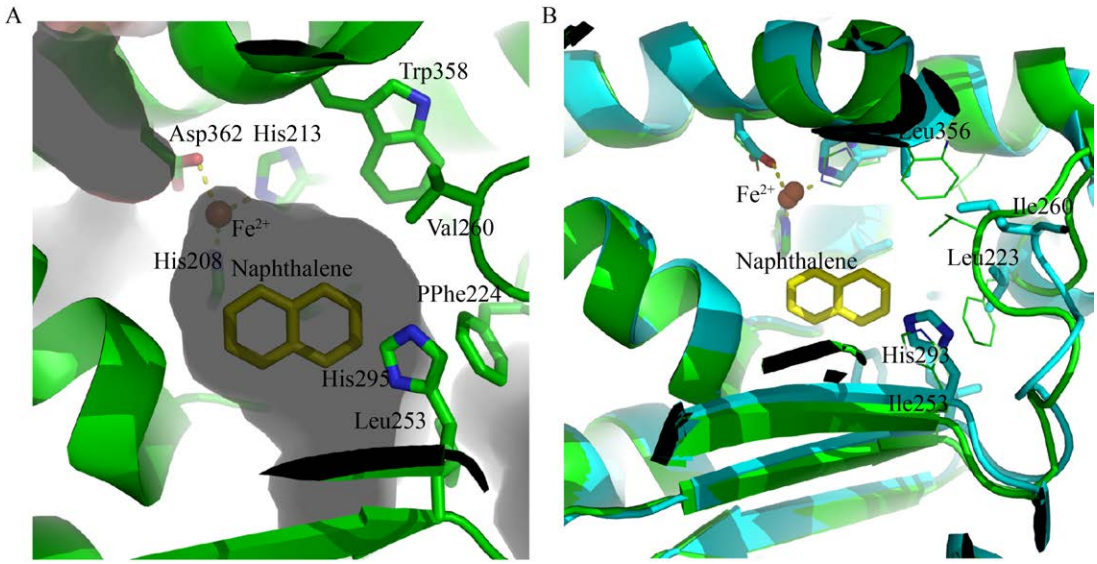


**图 5　RHOs 底物结合口袋**

Fig. 5　The ligand binding pocket of RHOs. (A) The ligand binding pocket of NDO (PDB ID: 1O7G) from *Pseudomonas* sp. NCIB 9816-4. The naphthalene molecule was shown in yellow and some important residue in green. (B) Comparison with RHOs from *Sphingomonas* CHY-1 (Blue, PDB ID: 2CKF) and from *Pseudomonas* sp. NCIB 9816-4 (green). Residues Leu223 and Ile260 with smaller amino acid side chains indicated the possibility of that *Sphingomonas* CHY-1 RHOs bind PAHs molecules with larger molecular weight.

## 5　一些针对 RHOs 和多环芳烃的研究方法

### 5.1　环境中 RHOs 的筛选

用唯一碳源的无机盐培养基从污染环境中富集分离多环芳烃降解菌株是普遍采用的方式。通过污染物压力筛选能产生与多环芳烃降解相关的微群落，进而研究其中的 RHOs[117]。环境中还存在着非常多的不可培养微生物发挥降解作用，因此研究人员利用分子生物学的方法来筛选和检测环境中的 RHOs，如建立 RHOs 的加氧酶组分的 α-亚基基因序列数据库，以保守的 Rieske 中心的核酸序列，设计高度特异性的引物以进行 RHOs 的丰度或多样性等的研究[118]。一系列基于保守区域引物的技术被应用，如聚合酶链式反应 (Polymerase chain reaction，PCR)[119]、利用双加氧酶探针进行 Southern 杂交实验[120]、多重 PCR 技术等来鉴定和检测芳香族双加氧酶基因，以及利用定量 PCR 扩增技术量化环境样品中的芳香族分解代谢基因[121]。检测到环境中存在的 RHOs 后，通过构建宏基因组文库，如以 Fosmid、Cosmid、BAC 为载体的文库构建，可以获取到相关 RHOs 的全长序列[122]，能够对其功能进行研究及表征，扩展对于未培养微生物中 PAH 降解基因的了解，更能够扩大对于 RHOs 的认知，找到更多功能强大的 RHOs 来用于场地或相关环境的修复。

### 5.2　大肠杆菌异源表达-体内活性测定

RHOs 功能表征的一个研究思路是将 RHOs 的双加氧酶组分、电子传递组分分别克隆到不同质粒上，转到同一宿主中进行诱导表达，若要检测这些基因是否正确表达产生了有降解活性的 RHOs，则可以采用靛蓝筛选法[123]、高效液相色谱 (HPLC) 或气相色谱 (GC) 检测代谢物，使用氧气电极检测实时活性[124]。

其中靛蓝筛选法是最为方便快捷的方法，萘双加氧酶型 RHOs 的特征是吲哚向靛蓝转化，添加不高于 4 mmol/L 的吲哚 (高于 4 mmol/L 对细

胞有毒性) 能使得菌株中的活性萘双加氧酶(NDO) 生成靛蓝[125]，大肠杆菌细胞可以利用色氨酸酶从色氨酸合成吲哚，所以含有 RHOs 的克隆不需要添加吲哚即可验证在大肠杆菌中的活性[126]，从而作为筛选 NDO 的表征。而 HPLC 或 GC-MS (质谱) 联用是最常用的多环芳烃降解产物的检测和鉴定方法[3,93,127]。但该方法操作较为复杂，要对代谢产物经过反复萃取-旋蒸浓缩-干燥除水-重溶-过滤等步骤，不适合高通量的筛选，但是能较为精确地定量。基于高碘酸盐荧光检测 RHOs 产生的顺式二醇代谢物的方法是一种新的筛选方法，其采用高碘酸钠氧化顺式二醇代谢物转化为相应的二醛，用以产生可检测的分析物[128]。此方法与传统方法相比优势在于能够高通量地筛选双加氧酶或者有羟基化多环芳烃能力的突变体双加氧酶。

### 5.3 重组蛋白的体外活性与底物结合

RHOs 的体外酶活测定需要完整的酶组分才能进行，因而需要将加氧酶组分和 Fd、FdR 电子传递组分都进行纯化表达，得到纯度较高的蛋白质，通过在 340 nm 处 NADH 的消耗来测定酶活性[61]，或者利用氧电极测定耗氧速率[129]。此外，差示扫描荧光法 (Differential scanning fluorimetry，DSF) 是一种基于蛋白质的热稳定性原理高通量筛选蛋白质与配体相互作用的方法，在其他蛋白质的配体筛选中已经被广泛使用。此种方法结合上文提到的高通量的荧光检测方法来检测 RHOs 与多环芳烃的混合物孵育后的产物中是否有顺式二醇代谢物，不失为一种简单的检测方法，为 RHOs 的底物筛选提供了一种高通量的方式。

而重组蛋白测活的难点在于 FdR 的表达存在问题，大部分重组蛋白表达为包涵体的形式，而这个问题不能通过改变宿主菌株或在诱导过程中降低温度来解决[43]，睾丸酮丛毛单胞菌 *Comamonas testosterone* B-356 联苯双加氧酶的 FdR 组分表达时也遇到了相同的问题，最后使用 QIAGEN His-Binding Kit 纯化得到能用于实验的蛋白量[130]。从类产碱假单胞菌 *Pseudomonas pseudoalcaligenes* KF707 中克隆表达联苯双加氧酶的 FdR 组分时，用了一个含有分子伴侣 *groELS* 基因的质粒 pKY206 成功表达了可溶性的 FdR 蛋白[131]。*Sphingomonas* sp. strain CHY-1 的 FdR 组分克隆到 pET15b 载体上转化到大肠杆菌 BL21(DE3) 中进行体内活性测定时，发现比没有 Fd、FdR 组分的菌株对菲的降解率增加了 35 倍，说明 FdR 在大肠杆菌中正确合成并发挥作用[132]，但后期纯化时只能获得少量的该酶，也不能进行大量的表达，因而更换了其他菌株中更为稳定的 FdR 进行实验，并不影响电子传递作用[43]。以 *Pseudomonas putida* F1 中的甲苯双加氧酶进行了实验验证，在更换 Fd 的情况下，不更换 FdR 能获得原来活性的 56%，而更换合适的 FdR 也能使得活性达到原来的 38%[84]。因而当 FdR 不能正确表达时，可以使用分子伴侣辅助 FdR 表达，也可尝试更换其他来源的 FdR 电子传递组分进行酶活测定。

### 5.4 分子模拟

通过同源建模、分子对接模拟分析多环芳烃与双加氧酶活性中心的相互作用机理从而解释实验现象是一种比较普遍的方法[23]。研究 RHOs 底物特异性的方法已经不仅仅局限于实验结果，从计算机的分子模拟中可获得底物和酶的结构信息，并可以解释生化实验中的一些现象，为实验提供参考性的帮助，而且通过对接分析预测的大多数底物是可以通过实验验证的[126]。目前的同源建模可以采用 Geno3D、SWISS-MODEL、PHYRE、MODELLER 和 Schrödinger。建模的结果可通过 PROCHECK[59]获取到 Ramachandran 图来评分，或者通过 VADAR 软件评估单个残基和整个蛋白质的关键结构参数来评估模型的质量，结构错误可通过 WHAT-CHECK 进行分析[126]，氨基酸序列与其 3D 原子模型之间的相容性通过 VERIFY-3D

进行评估[59,129]。MOLE 2.0 识别从溶剂区域通向活性部位的可能通道[133]，POCASA 1.0 可预测催化口袋的区域[126]，分子对接的软件可使用 AutoDock Vina[134]或者 Sybyl7.3 中的 Surflex 模块，用于探索 NDO 活性位点与配体之间的相互作用机理，Surflex 可以有效地减少假阳性结果的百分比[59]。分子模拟在 RHOs 的研究中是一个非常好的工具，它能够在获得蛋白质与多环芳烃复合物结构之前推测蛋白与底物的相互作用。对 RHOs 进行设计改造以产生更有效的酶活，适应环境实际需求来进行环境修复方面，分子模拟是非常重要的[135]。

## 6 潜在应用与展望

Rieske 非血红素铁环羟基化双加氧酶在多环芳烃降解中发挥着重要的作用，它是唯一能催化多环芳烃形成顺式二氢二醇的酶，与多环芳烃污染修复有着紧密关联。其一，根据 RHOs 的保守性，能对环境中存在的多环芳烃降解菌株进行定量及筛选，结合宏基因组学相关技术[122]，能够获取到更多不可培养微生物的基因信息，不但丰富对于不可培养微生物的认识，更能够获取到新的高效降解多环芳烃的基因。其二，RHOs 可以作为一种多环芳烃污染场地的指示物，针对 RHOs 保守区域制备相应的引物即可监测受污染场所的芳烃降解菌株的种群丰度[118]。其三，在长期污染的环境中，RHOs 是可以在菌群中水平转移的，从而使得微生物能够适应这种极端恶劣环境[50]。人工模拟自然条件下的水平转移，可获得含有快速进化的高效 RHOs 的微生物，将其应用到污染环境中，能够更好地适应环境以降解污染物。其四，伴随着合成生物学的发展，通过分子模拟来设计和改造 RHOs，结合定向突变技术[101]，能够获得更多有益的 RHOs 的突变体，扩宽 RHOs 的底物催化范围，一种底物范围广、高效而且稳定的酶制剂或人工微生物对多环芳烃污染治理有着潜在的应用价值。其五，多环芳烃诸多的降解基因簇也被用于构建基因工程微生物 (Genetically engineered microorganisms，GEMs)，如 *nah-like* 基因簇等，GEMs 可被用来清理污染场地，提高污染物的去除能力及范围[136]。目前，已经有一些 RHOs 或利用基因工程技术改造过的 RHOs 被申请专利，未来有潜力被用来处理受到有毒或致癌芳香族化合物污染的土壤、湿地和污水[137-139]。对于多环芳烃污染场地的生物修复来说，RHOs 有着广阔的应用前景。

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