

# Chapter 7

## Genetic Adaptations of Bacteria for Metabolism of Polycyclic Aromatic Hydrocarbons

Vidya de Gannes and William J. Hickey

**Abstract** Polycyclic aromatic compounds (PAH) are a family of chemicals containing two or more fused benzene rings, which present ecotoxicological concerns ranging from acute toxicity in aquatic organisms to carcinogenesis in mammals. In contrast, microbial ecotoxicology of PAH centers on metabolic activities that enable utilization of PAH as growth supporting substrates. This chapter focuses on PAH biodegradation by aerobic bacteria, and examines characteristics that are important in PAH metabolism at three levels: enzyme systems that mediate catabolism and carbon assimilation, regulatory circuitry that controls expression of catabolic enzymes and cell structures and physiological activities that affect PAH uptake. The goal is to present a holistic view of the organisms and their biology that is relevant to PAH biodegradation. Of these areas, catabolism is the most developed and key enzymes and mechanisms have been elucidated. However, comparatively little is known about the regulatory systems that control expression of these enzymes. Uptake from the environment is the single most important factor affecting PAH degradation and while cellular characteristics that affect uptake are known, the process is still largely a “black box” and mechanistic details are lacking, especially regarding molecules that may facilitate PAH access. Mechanisms of regulation and uptake remain areas in need of future research. Future work should also focus on moving beyond studies of individual organisms, and gaining an understanding of PAH biodegradation processes operating within microbial consortia.

**Keywords** Polycyclic aromatic hydrocarbons • Biodegradation • Genetic adaptations • Catabolic pathways • Exopolymeric substance • Ring hydroxylating dioxygenase • Regulatory elements • Biomarkers

---

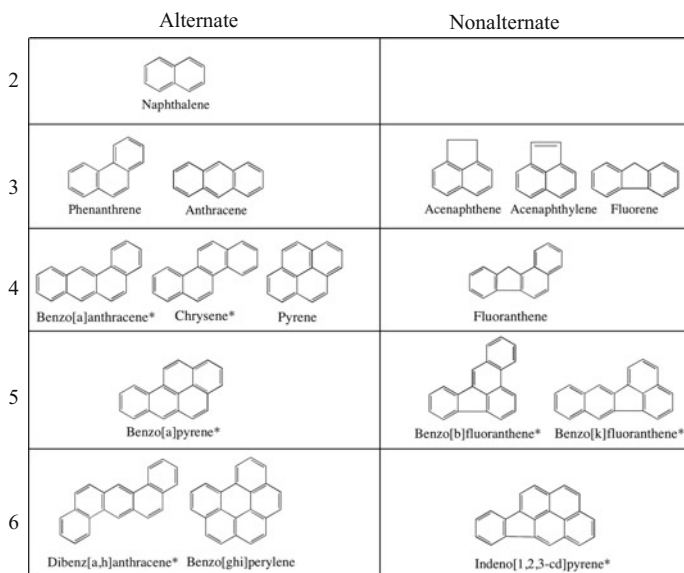
V. de Gannes  
Department of Food Production, University of the West Indies,  
St. Augustine, Trinidad and Tobago

W.J. Hickey (✉)  
O.N. Allen Laboratory for Soil Microbiology, Department of Soil Science,  
University of Wisconsin, Madison, WI 53706, USA  
e-mail: wjhickey@wisc.edu

## 7.1 Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a family of compounds, members of which contain two or more benzene rings fused in linear, angular or clustered geometries (Fig. 7.1). While benzene rings form the structural core of PAHs, some of these chemicals, termed non-alternate PAH (Fig. 7.1), are composed of benzene rings fused to other non-benzene structures (Harvey 1997). The PAHs of environmental concern are categorized as low-molecular weight (LMW, 2–3 rings) or high-molecular weight (HMW, 4–6 rings) and each group has different toxicological impacts on macrofauna; LMW PAHs may have significant acute toxicity to aquatic organisms, while some of the HMW are potential carcinogens for both wildlife and humans. Based upon demonstrated or suspected potential to cause human cancer, the US EPA identified a subset of 16 PAH as Priority Pollutants (Fig. 7.1) upon which the majority of research has focused.

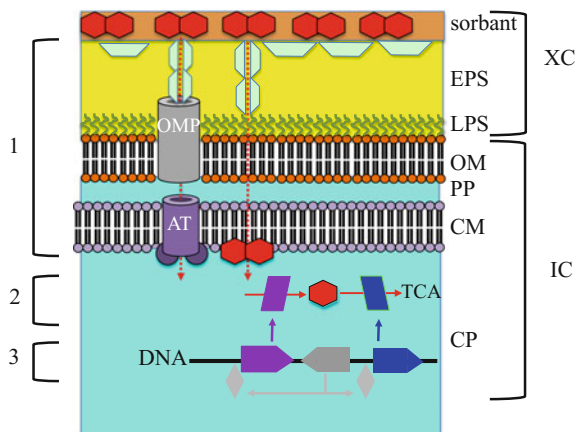
PAHs are released into the environment by a variety of natural and anthropogenic processes (Harvey 1997). But, point sources from the latter, primarily spills or improper disposal of hydrocarbon liquids containing PAHs, represent the main ecotoxicological concerns, and cases where microbial activity is of greatest interest *vis a vis* bioremediation. Marine oil spills, such as those of the *Deepwater Horizon*, garner much attention and can inflict widespread ecological damage. But, while the



**Fig. 7.1** Structures of 16 PAH identified by the US EPA as priority pollutants. *Values on the left* indicate the number of rings composing the PAH. The PAH are also sorted by structure as alternate or non-alternate. Compounds classified by the US EPA as probable human carcinogens are indicated by *asterisks*

fate of PAHs released in crude oil is a concern, the PAH content of oil is relatively small, ranging from 0.2 to 7% (Harvey 1997). Oil-derived fuels (e.g., gasoline, diesel) are wide spread environmental pollutants, but typically have a PAH content that is less than the oil parent material. In contrast, in liquids derived from coal (e.g., coal tar and creosote), PAHs comprise the largest single class of chemicals (>50%) and thus pose significant point sources of PAHs (Birak and Miller 2009; Brown et al. 2006; Wehrer et al. 2011). Coal tar and creosote are primarily threats to terrestrial ecosystems (soil, ground water, lakes, rivers) as they were associated with land-based operations; coal tar is a legacy contaminant at former sites of manufactured coal gasification plants and creosote (a derivative of coal tar) is a legacy contaminant at wood- and lumber-treatment facilities. Thus, PAH ecotoxicology and microbial ecotoxicology is relevant to a wide-range of contaminated environments, particularly at sites impacted by coal tar or creosote.

Traditional ecotoxicology of PAHs focuses on the deleterious effects of these chemicals on humans and wildlife. In contrast, microbial ecotoxicology of PAH includes deleterious impacts on microbes as well as positive interactions of PAH with these organisms. The latter of these two dimensions has been the dominant focus in this area, and centers upon the ability of microbes to consume PAH and obtain carbon and energy to support growth. Microbial biodegradation of PAH is therefore a potentially important ecosystem service and can mitigate exposure of humans and wildlife to these chemicals. The scope of research on microbial ecotoxicology of PAH ranges from single organism studies focused on elucidation of the molecular mechanisms that enable growth on of PAH, to community-scale investigations seeking to determine associations between the diversity, abundance or activity of PAH-degraders and PAH contamination. A variety of microbes and microbial processes are potentially operative in PAH biodegradation, which includes transformations mediated by bacteria and fungi in aerobic environments and bacterial transformations in anaerobic systems (Bouchez-Naitali et al. 2008; Doyle et al. 2008; Fernandez-Luqueno et al. 2011; Jain 2015; Johnston and Johnston 2012; Peng et al. 2008; Seo et al. 2009; Van Hamme et al. 2003). This chapter will focus on PAH biodegradation by aerobic bacteria, which has been the subject of the vast majority of research in the microbial ecotoxicology of these chemicals. Genetic adaptations that are key in enabling bacteria to grow on PAH will be examined at three levels (Fig. 7.2): (1) enzymes that mediate catabolism and carbon assimilation, (2) regulatory circuitry that controls expression of catabolic enzymes and (3) cellular structures that affect PAH uptake. All of these characteristics are endowed by an organism's genotype, and thus as a group represent the genetic adaptations that affect its capability to degrade PAH. A holistic view such as this enables recognition of the range of genetic adaptations that affect bacterial degradation of PAH, and an appreciation of the interactions between adaptations at different levels that collectively result in PAH biodegradation. For community-level studies, elucidation of genetic adaptations yields insights into genes that may be useful as biomarkers of PAH-degrading bacteria. Moreover, an appreciation of the interactions between a biomarker and other components of a cell that contribute to the biodegradation process is equally important to understand the extent to which



**Fig. 7.2** Overview of cellular structures and processes affecting PAH biodegradation. *Numbers* listed along the *left* indicate key cellular components: *1* structures involved in uptake, *2* catabolic enzymes and *3* regulatory systems controlling expression of catabolic enzymes. These components are grouped (*right*) as either extracellular (XC) and intracellular (IC). The diagram depicts a model *Proteobacterium* possessing exopolymeric substance (EPS), lipopolysaccharide (LPS), outer membrane (OM), periplasm (PP), cytoplasmic membrane (CM) and cytoplasm (CP). The cell is growing on the surface of an environmental sorbent (organic matter or hydrocarbon liquid) and shows the PAH diffusing through the extracellular and intracellular space to reach the catabolic enzymes of the cytoplasm. Structures that may facilitate diffusion are molecules creating hydrophobic domains in the polysaccharide matrix of the EPS/LPS (e.g., biosurfactants, lipids, proteins, shown as green structures in EPS) and OM proteins (OMP). The possibility for active transport (AT) across the CM is also indicated. Otherwise, PAH diffusion from the sorbent to the cytoplasm is driven solely by concentration gradients and partitioning behavior of the PAH across the different EC and IC regions. The catabolic enzymes are depicted as grouped into the upper pathway (*purple rectangle*) that transforms the PAH to a monoaromatic acid and lower pathway (*blue rectangle*) that completes metabolism. The operons encoding enzymes of the upper and lower pathways are depicted as *purple* and *blue block arrows*, and a regulatory element controlling expression of the operon is depicted in *gray*

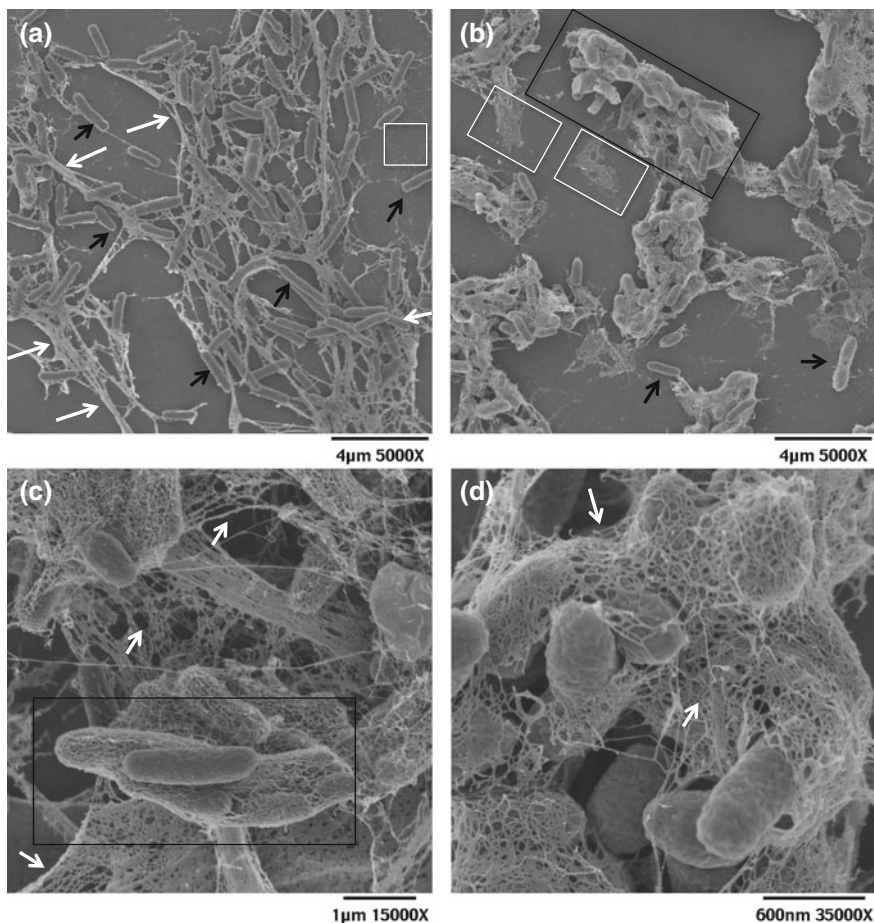
inferences can be drawn about interactions between PAH and communities of PAH-degraders based upon the biomarker analysis. Thus, while this chapter primarily focuses on the cellular systems, examples of applications of PAH biomarkers to community analysis will also be examined.

## 7.2 PAH Catabolic Pathways

Bacterial pathways for PAH catabolism have been examined extensively, and are the subject of a number of reviews to which the reader is referred for details (Bouchez-Naitali et al. 2008; Doyle et al. 2008; Peng et al. 2008; Seo et al. 2009). Our understanding of bacterial genetics and metabolism underlying PAH biodegradation is rooted in the studies of naphthalene done with the archetypical

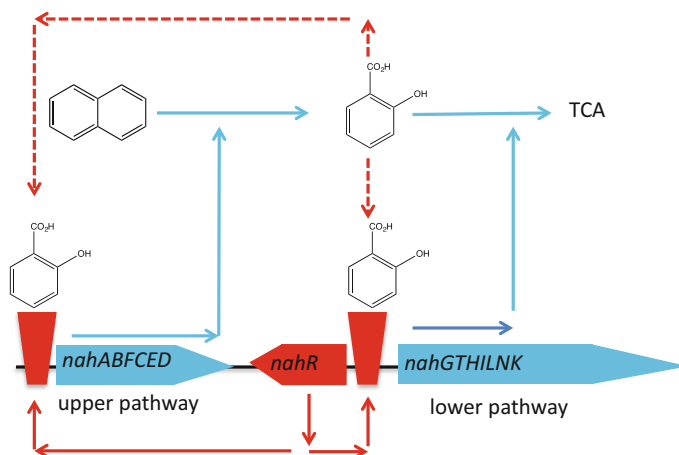
plasmid pNAH7 from *Pseudomonas putida* G7 (Yen and Serdar 1988). A key concept formulated from those studies is the physiological and genetic division of PAH catabolism into “upper” and “lower” segments (Schell et al. 1990). The upper pathways are composed of the initial ring hydroxylating dioxygenases (RHD) and subsequent enzymes that collectively achieve fission of one of the aromatic rings, release of carbon (e.g., pyruvate or other aliphatic acid) and generation of an aromatic acid, which is the starting substrate for the lower pathway (Fig. 7.3). In the NAH7 model, naphthalene is metabolized via the upper pathway to salicylate and an adjacent operon encodes the lower pathway for salicylate catabolism (Fig. 7.3). Both operons are positively regulated by salicylate (Fig. 7.4). While the NAH7 model has been useful to establish many basic principles of bacterial PAH, there are a number of important differences between it and other PAH pathways. For example, instead of salicylate, upper pathways may yield *o*-phthalate, gentisate or protocatechuate, and which of these compounds is produced depends upon the parent PAH, variations in regiospecificity of the RHD and the occurrence of upper pathway enzymes mediating transformations of key intermediates. Differences between upper pathways in the aromatic acid product are significant, because salicylate is thus far the only compound conclusively identified to regulate transcription of the cognate operons. Thus, for pathways lacking salicylate as an intermediate, compounds that serve this critical function are unknown. Also, while upper- and lower-pathway operons on the NAH7 plasmid are in relatively close proximity, the two operons may be chromosomally encoded in many PAH degraders, and in these cases it is not uncommon for the upper- and lower-pathway operons to be encoded at loci distant from each other (Shetty et al. 2015; Tang et al. 2011; Cao et al. 2015; Choi et al. 2015; Dong et al. 2014; Kallimanis et al. 2011; Kim et al. 2008; Kwak et al. 2014; Lai et al. 2012; Li et al. 2016; Maeda et al. 2013; Wang et al. 2014a, b, 2015, 2016; Zhang et al. 2012; Messina et al. 2016).

The cornerstone of PAH degradation pathways are the RHD (Rieske non-heme iron dioxygenase; E.C. 1.14.12), which activate PAH for catabolism via reductive dihydroxylation. RHD consist of an NADH-dependent reductase, a [2Fe-2S] ferredoxin and a terminal oxygenase. The latter is comprised of two separate polypeptides, a large subunit ( $\alpha$ -subunit, RHD $\alpha$ ) containing [2Fe-2S] and mononuclear Fe(II) at the active site, and small subunit ( $\beta$ -subunit, RHD $\beta$ ) that appears to serve a structural function (Parales and Ju 2011). The reductase and ferredoxin transport electrons to the  $\alpha$ -subunit, which inserts molecular oxygen into the substrate to give *cis*-dihydrodiols, which are subsequently transformed to diols by a dehydrogenase (Fig. 7.5). Most PAH RHD are classified as lateral dioxygenases and add oxygen to two adjacent carbon atoms of an aromatic ring PAH (Fig. 7.5). A smaller group of PAH RHD are categorized as angular dioxygenases, and act on non-alternate PAH, adding oxygen to the carbon atom of an aromatic ring and to an adjacent carbon atom bonded to a non-benzene structure (Nojiri et al. 2001; Schuler et al. 2008). To date, the best characterized angular dioxygenase active in PAH metabolism is the FlnA enzyme in the fluorene-degrader *Sphingomonas* sp. LB126 (Schuler et al. 2008).

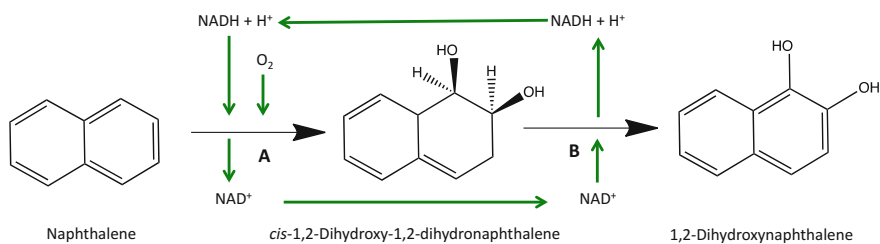


**Fig. 7.3** Production of exopolymeric substance (EPS) by a phenanthrene-degrading biofilm of *Delftia acidovorans* Cs1-4. Cells of *D. acidovorans* Cs1-4 were collected on 0.22  $\mu\text{m}$  polycarbonate membranes, and then floated on the surface of a mineral salt medium containing phenanthrene crystals (1 mg/mL). The filters were incubated statically, and growth examined by scanning electron microscopy (SEM) after 2 d (Panel **a**) or 7 d (Panels **b–d**). Sample preparation for SEM imaging involves dehydration, and thus the originally hydrated EPS appears as an extensive network of web-like strands (white arrows) connecting and surrounding cells (black arrows) as well as coating the membrane surface (white boxes). In the early stage (Panel **a**), cells are distributed primarily as a dispersed monolayer, which progress to form multilayer cell clumps (Panel **b**, black box). In later stages, the EPS serves to encase groups of cells (Panel **c**, black box)

PAH RHD are broadly distributed across microbial taxa, and comparisons of similarities in PAH-RHD $\alpha$  amino acid sequence have revealed distinct PAH-RHD $\alpha$  families (Habe and Omori 2003; Moser and Stahl 2001), the phylogenies of which generally follow those of the host (Table 7.1). Thus, PAH-RHD $\alpha$  of Proteobacteria and Gram-positive bacteria (*Actinobacteria*) separate into two distinct clades and



**Fig. 7.4** PAH degradation based upon the naphthalene-NAH7 model showing catabolic and regulatory systems in blue and red, respectively. Two operons encode catabolic enzymes that transform naphthalene to salicylate (*upper pathway*) and salicylate to TCA intermediates (*lower pathway*). The *nahR* gene encodes a LysR-type transcriptional repressor-activator that binds to promoters upstream of both the upper- and lower-pathways and represses transcription allowing only a basal level of expression. Binding of salicylate to NahR at the promoters induces a conformational change in DNA-bound NahR that enables transcription to occur



**Fig. 7.5** Mechanism of reductive ring dihydroxylation by an example PAH RHD, and coupling of electron flow with subsequent dehydrogenation of the dihydrodiol intermediate. The enzyme catalyzing *Step A* is Naphthalene dioxygenase while *Step B* is mediated by 1,2-Dihydroxy-1,2-dihydronaphthalene dehydrogenase

within each of these broad groups PAH-RHD $\alpha$  subclades are associated with particular bacterial classes. In the Proteobacteria, there are at least five PAH-RHD $\alpha$  families (Table 7.1), the largest of which is the NahAc family that is generally associated with aquatic/terrestrial bacteria of the Gammaproteobacteria, primarily pseudomonads. Notably, the PAH-RHD $\alpha$  of marine gammaproteobacterial PAH-degraders, such as *Cycloclasticus*, is more closely related to the PAH-RHD $\alpha$  of the alphaproteobacterial Sphingomonads than it is to the NahAc group (Kasai et al. 2003). Other proteobacterial PAH-RHD $\alpha$  families are NagAc, PhnAc<sub>AFK2</sub>, PhnAc<sub>RP007</sub> and AhdA1/BphA1. The NagAc family is closely related to the NahAc



**Table 7.1** Families of PAH upper pathway ring hydroxylating dioxygenases

Family	Class or genera	Lower pathway
<i>Proteobacteria</i>		
NahAc	Gamma-/Beta-Proteobacteria	Salicylate
NagAc	Betaproteobacteria	Gentisate
PhnAc <sub>AFK2</sub>	Betaproteobacteria	<i>o</i> -Phthalate
PhnAc <sub>RP007</sub>	Betaproteobacteria	Salicylate
AhdA1/BphA1	Alphaproteobacteria (Sphingomonads)	Salicylate or <i>o</i> -Phthalate
<i>Actinobacteria</i>		
NidA/PdoA1	<i>Mycobacterium</i>	<i>o</i> -Phthalate
NidA3/FadA1	<i>Mycobacterium/Terrabacter</i>	<i>o</i> -Phthalate
NarA	<i>Rhodococcus</i>	Gentisate
PdoA2/PhdA	<i>Mycobacterium/Nocardia</i>	<i>o</i> -Phthalate

group and characteristically produces gentisate, rather than salicylate, as the lower pathway substrate (Nag = Naphthalene to gentisate (Fuenmayor et al. 1998)). The PhnAc<sub>AFK2</sub> family is named after bacterium in which it was first identified, *Alcaligenes faecalis* AFK2 (Kiyohara et al. 1982), and the “Phn” designation is used to indicate that PAH-degraders with a PhnAc<sub>AFK2</sub> genotype are typically limited to phenanthrene as the sole PAH utilized to support growth. The PhnAc<sub>AFK2</sub>-type PAH-RHD $\alpha$  is also characteristically linked to upper pathways producing *o*-phthalate (Kiyohara et al. 1982). The PhnAc<sub>RP007</sub> family was discovered in *Burkholderia* sp. RP007 (now *Paraburkholderia sartisoli* RP007 (Laurie and Lloyd-Jones 1999)) and despite the similarity in name to PhnAc<sub>AFK2</sub>, PhnAc<sub>RP007</sub> and PhnAc<sub>AFK2</sub> genotypes differ in that the former: (1) utilize naphthalene as well as phenanthrene to support growth, and (2) produce salicylate from the upper pathway rather than *o*-phthalate (Laurie and Lloyd-Jones 1999). The PAH-RHD $\alpha$  of Sphingomonads (AhdA1/BphA1) may be associated with upper pathways generating either salicylate or *o*-phthalate (Waigi et al. 2015). Actinobacteria are represented by four groups PAH-RHD $\alpha$  groups, NidA/PdoA1, NidA3/FadA1/NarA and PdoA2/PhdA and are generally associated with upper pathways that generate *o*-phthalate (Table 7.1).

A key difference between PAH-RHD are physical characteristics of the active site, which affect the type and range of PAH on which the enzyme will act and funnel to a productive catabolic (lower) pathway. Proteobacterial RHD are generally most active with low molecular weight PAH ( $\leq$  three rings) with high molecular weight PAH (HMW PAH,  $\geq$  four rings) transformed at comparatively low levels (Baboshin et al. 2014; Jouanneau et al. 2006). In contrast, RHD of the actinobacterial NidA and NidA3 families show higher activity towards HMW PAH, than they do with LMW PAH (Kweon et al. 2010; Krivobok et al. 2003). The preference for LMW- versus HMW-PAH can be explained at least in part by differences between RHD in the substrate-binding pocket, which is larger in NidA/NidA3 enzymes than in proteobacterial RHD (Kweon et al. 2010). Geometry



of the active site also effects regioselectivity of dihydroxylation. For example, the NidAB and NidA3B3 homologs from *Mycobacterium vanbaalenii* PYR1 transformed a range of PAH, but displayed the highest regioselectivity with the preferred substrate, pyrene and fluoranthene, respectively (Kweon et al. 2010). Regioselective dihydroxylation by RHD is important in yielding products utilized as substrates for the subsequent enzymes of the upper pathway, and ultimately yield carbon to central metabolism. The RHD reactions consume electrons (Parales and Ju 2011) and thereby impose a negative energy yield, unless coupled with a subsequent dehydrogenase that recover electrons (Fig. 7.4). Thus, coordination between the RHD and dehydrogenases in the range of substrates utilized by each is important to minimize energy loss. Nevertheless, PAH-RHD exhibit varying degrees of non-selectivity, and will hydroxylate PAH that are not utilized as growth supporting substrates in a process termed cometabolism. For an individual organism, cometabolism is negative as it results in energy loss, and oxidation of PAH that are not growth-supporting may compete with that occurring with PAH that do sustain growth. But, from a community perspective, cometabolism may be beneficial as it could generate metabolites (hydroxylated PAH) that maybe utilized by commensal organisms.

While the activities of RHD and dehydrogenases are important first steps, productive metabolism of a PAH is equally dependent upon the collective activities of the upper pathway enzymes. For bacteria that utilize more than one PAH as a growth substrate, or generate multiple pathways for any given PAH (Seo et al. 2012), the upper pathway would include a combination of core enzymes with broad specificities, which ultimately couple with enzymes producing key aromatic acid intermediates. For example, in *Pseudomonas putida* NCIB 9816, a common set of upper pathway enzymes enables growth on naphthalene, fluorene and phenanthrene (Yang et al. 1994). Likewise, in *Sphingomonas paucimobilis* EPA505 mutational analysis indicated that catabolism of naphthalene, anthracene, and phenanthrene was mediated by a common set of enzymes (Story et al. 2001).

As noted above, the monoaromatic compounds that link the upper and lower pathways are limited to salicylate, gentisate or *o*-phthalate. Pathways for catabolism of these compounds are wide spread in bacteria, and occur in many organisms that are not PAH degraders. Thus, these systems are not unique to PAH degraders, and the reader is referred to reviews for detailed descriptions (Albaiges 2013; Diaz et al. 2013; Ladino-Orjuela et al. 2016; Vamsee-Krishna and Phale 2008). The broad distribution of the lower pathways in microbial communities may present a mechanism for cooperative metabolism, wherein organisms that mediate primary degradation (upper pathway) secrete metabolites that are utilized by commensals. A number of PAH-degrading consortia have been reported, and commensalism is generally proposed as a mechanism supporting the stability of these diverse assemblages although the actual metabolites that might be exchanged are undetermined (Boonchan et al. 2000; Kuppusamy et al. 2016; Lafortune et al. 2009; Stach and Burns 2002; Vinas et al. 2005). Unraveling metabolic interactions occurring within consortia remains a goal of future research.

### 7.3 Regulation of PAH Gene Expression

Regulation of PAH gene expression is a key aspect in controlling the biodegradation activities of bacteria in the environment and also important in applied technologies such as strain engineering, development of biosensors and devising strategies for bioremediation. Regulation is multi-tiered system: expression of enzymes effecting PAH degradation is controlled at the level of the cognate genes by regulatory proteins (transcription factors) specific for the catabolic operon, which interact with RNA polymerase (RNAP) to activate or de-repress gene expression. These genes may also be subject to regulation by elements that operate globally, and integrate carbon assimilation from PAH into the overall metabolism of the cell. For example, while most PAH upper pathway operons are transcribed by a  $\sigma^{70}$ -RNAP holoenzyme (e.g., bind to  $\sigma^{70}$ -dependent promoter) others are expressed with the  $\sigma^{54}$ -RNAP complex and require interaction with global regulatory elements (Table 7.2).

Genes encoding each of the PAH-RHD families tend to be associated with a specific type of regulatory element from either the LysR, TetR, MarR, GntR or NtrC classes. However, the only regulatory system that has been studied in detail in association with PAH metabolism is that of NahR, a LysR-type element that regulates expression of the NahAc family RHD (and NagAc family, Table 7.2) and the associated genes of the upper and lower pathways (Park et al. 2002). Thus, most of the regulatory systems that control expression of enzymes for PAH metabolism are uncharacterized. This is a key knowledge gap in our understanding PAH biodegradation, and it's unknown how differences in these systems may impact growth of PAH-degraders in the environment. This is significant as PAH-degraders with LysR systems represent only one segment of environmental communities of PAH-degraders (*nah* and *nag* types, Table 7.2), which often do not appear to be the most abundant genotype (see section below on microbial ecology and PAH biomarkers). Thus, information about regulatory elements other than LysR would benefit our understanding of PAH degradation at the cellular and community levels.

In the NahR system, the gene encoding the regulator (*nahR*) is immediately upstream of the of lower pathway operon (Fig. 7.4). NahR is continuously bound to a promoter between *nahR* and the downstream operon ( $P_{sal}$ ), as well as to a promoter upstream of the upper pathway operon ( $P_{nah}$ ) and its presence allows only a low level of a constitutive expression. Repression is lifted when the promoter-bound NahR binds salicylate (the effector), inducing a conformational shift that enables RNAP binding and full level transcription of both the upper- and lower-pathway operons (Schell et al. 1990; Huang and Schell 1991; Lonneborg and Brzezinski 2011; Park et al. 2005; Wilkinson and Grove 2006; Maddocks and Oyston 2008; Schell 1985). The Nag RHD family is also associated with an NahR ortholog (NagR), and although the product of Nag upper pathway is gentisate rather than salicylate, only the latter compound induces expression (Jones et al. 2003).

In contrast to the Nah model, regulation of PAH degradation by bacteria possessing a PhnAc<sub>AFK2</sub> family RHD appears to be more complex, as the upper

**Table 7.2** Summary of regulatory elements associated with families of PAH ring hydroxylating dioxygenases alpha subunits (PAH-RHD $\alpha$ ) and upper pathway operons

Class	Action <sup>a</sup>	<i>Proteobacteria</i>		<i>Actinobacteria</i>		Effector	Promoter	Binding Site <sup>d</sup>	Other factors
		PAH-RHD $\alpha$ <sup>b</sup>	Gene or locus <sup>c</sup>	PAH-RHD $\alpha$	Gene or locus				
LysR	A/R	NahAc, NagAc, PhnAc <sub>RP007</sub>	<i>nahR</i> , <i>nagR</i> , <i>phnS</i>	–	–	Salicylate	$\sigma^{70}$	Operator	–
TetR	R	PhnAc <sub>AFK2</sub>	DelCs14_1747	–	–	Unknown	$\sigma^{70}$	Operator	–
MmrR	R	PhnAc <sub>AFK2</sub>	DelCs14_1753	NidA	<i>nidR</i>	Unknown	$\sigma^{70}$	Operator	–
GntR	R	–	–	NarA	<i>narR1</i>	Unknown	$\sigma^{70}$	Operator	–
AraC	A	–	–	PhdA	ORF12	Unknown	various	Various	Various
NtrC	A	AhdA1, PhnAc <sub>RP007</sub>	<i>ahdR</i> , <i>phnR</i>	NarA	<i>narR2</i>	Unknown	$\sigma^{54}$	UAS	IHF, ATP

<sup>a</sup>Abbreviations: A activator; R repressor

<sup>b</sup>See Table 7.1

<sup>c</sup>Name of gene encoding the indicated regulatory element. Locus or ORF is given when the cognate gene is unnamed

<sup>d</sup>Region to which regulatory protein binds

pathway includes both TetR and MarR regulatory elements while the lower pathway genes are associated with two additional regulators. The actinobacterial Nid family also has a gene encoding a MarR regulator (NidR (Kim et al. 2006)) and expression is presumably controlled by that system, although it has not been empirically substantiated. The potential functions of TetR or MarR in controlling *phn* or *nid* expression are unknown, but regulatory activities have been elucidated with other catabolic pathways. For the TetR group, most notably is CymR, which represses expression of *cym* genes encoding degradation of *p*-cymene. CymR represses *cym* expression by binding to an operator adjoining a  $\sigma^{70}$ -dependent promoter and blocking RNAP; repression is released when the CymR binds the effector, *p*-cumate a metabolite in the cymene pathway (not the parent compound, cymene), and subsequently disassociates from the promoter, allowing RNAP access and transcription. A TetR-type regulator has also recently been shown to negatively regulate testosterone catabolism in *Comamonas testosteroni*, however the effector molecules were not identified (Pan et al. 2015). MarR regulators generally act as transcriptional repressors in a manner similar to TetR, and bind operators nearby  $\sigma^{70}$ -dependent promoters thereby preventing RNAP binding and transcription of cognate operons (Alekhshun and Levy 1999). MarR regulation has been demonstrated for operons encoding phenylpropenoid catabolism in Firmicutes (CinR, *Butyrivibrio fibrisolvens* (Dalrymple and Swadling 1997)) and in Actinobacteria (PhdR, *Corynebacterium glutamicum* (Kallscheuer et al. 2016)). The identities of effectors were not conclusively determined, but MarR regulators in general may have an affinity for phenolic compounds (Alekhshun and Levy 1999) and, consistent with that concept, PhdR appeared to have an affinity for hydroxylated phenylpropenoids as ligands.

Other actinobacterial PAH genes are associated with GntR, NtrC or AraC regulatory elements (Table 7.2). The NarA family PAH-RHD associated with *Rhodococcus* and related genera is located downstream of two tandemly arranged transcriptional regulatory genes, *narR1* and *narR2*, which are predicted to encode proteins of the GntR and NtrC families, respectively (Kulakov et al. 2005; Larkin et al. 2005). The GntR-type regulators function like the above-described repressors in preventing effective binding of RNAP to a  $\sigma^{70}$ -dependent promoter, which is lifted by effector binding and consequent disassociation of the GntR from the operator. For NarR1 regulated operons, the identities of the effectors are unknown. However, GntR regulation has been established for biphenyl catabolism in *Acidovorax*, and in this case the effector ligand for the GntR repressor (BphS) is a product of biphenyl ring fission, 2-hydroxy-6-oxo-6-phenyl-2,4-dienoic acid (HODPA (Ohtsubo et al. 2001)). Similar metabolites (i.e., aromatic rings with extended aliphatic substituents) occur in PAH degradation pathways (Bouchez-Naitali et al. 2008; Peng et al. 2008, 2011; Seo et al. 2009; Vaidehi and Kulkarni 2012) and could be candidates as potential effectors.

NtrC-type regulators act as transcriptional activators, and are distinct from all of the above-mentioned transcription factors in that they: (1) interact with RNAP at  $\sigma^{54}$ -dependent promoters rather than  $\sigma^{70}$ -dependent promoters, (2) binds ATP as a cofactor in addition to an effector molecule, and (3) require a global regulatory

element (Integration Host Factor). NtrC-type factors are representatives of a broad group termed the prokaryotic enhancer binding proteins, which bind DNA in areas called upstream activating sequences (UAS) that are typically located a distance (*ca.*  $\geq 100$  bp) from the cognate  $\sigma^{54}$  promoter. NtrC activation of transcription is initiated when NtrC dimers in solution bind the effector inducing conformational changes that enable ATP binding, multimerization and subsequent binding to UAS. Integration Host Factor (IHF) binds at a site between the UAS and  $\sigma^{54}$  promoter, which induces bending and allows the DNA-bound NtrC complex to interact with RNAP bound to the  $\sigma^{54}$  promoter. NtrC then activates RNAP (coupled with ATP hydrolysis) by inducing a transition in RNAP conformation from closed to open, thereby enabling transcription.

NtrC regulatory proteins are associated with at least three PAH-RHD families: AhdA1/BphA1 (Khara et al. 2014), PhnA<sub>CRP007</sub> (Laurie and Lloyd-Jones 1999) and NarA (Kulakov et al. 2005). Details of NtrC regulation have not been developed for operons encoding PAH upper pathways, but the mechanisms have been explored in connection with catabolism of other aromatic compounds including phenol, hydroxybiphenyl, toluene and *m/p*-xylene (Diaz and Prieto 2000; Tropel and van der Meer 2004). In the pathways for which mechanisms of NtrC control have been defined, the parent compounds as well as some related aromatic compounds act as effectors (Diaz and Prieto 2000; Tropel and van der Meer 2004). The fact that neutral compounds such as toluene and xylenes act as effector ligands for NtrC distinguishes these regulators from all of the others discussed above, for which ligands appear limited to phenolics or amphiphilic compounds (e.g., HOPDA). For PAH operons, an affinity of NtrC for neutral ligands may be important, as it raises the possibility that the PAH parent compounds might act as inducers for their own catabolism. Notably, growth on naphthalene induced the *nar* operon in *Rhodococcus* (Di Gennaro et al. 2010), which is affiliated with an upstream NtrC-like regulatory element. But, it's unclear if naphthalene was an effector, as the cells were able to metabolize naphthalene to salicylate, which also induced the *nar* operon.

In contrast to the Nar family, actinobacterial Phd genes are associated with an AraC-type transcriptional activator (Pagnout et al. 2007). Mechanisms of AraC regulation that are specific to the *phd* operon are unknown, and are difficult to infer because these AraC elements vary widely in fundamental characteristics such as the types of promoters regulated ( $\sigma^{70}$  and others) and their dependence on other regulatory factors for maximal activity (e.g., catabolite repression protein (Gallegos et al. 1997)).

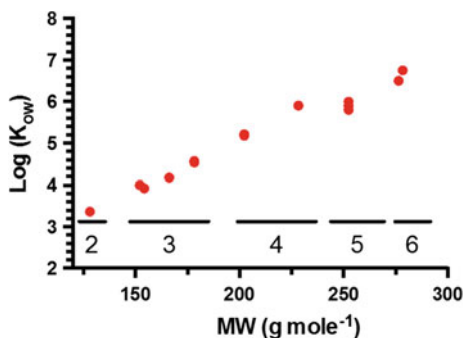
Understanding the mechanisms underlying regulation of genes encoding catabolism of PAH has practical importance in the development of bioremediation strategies. For example, supplementation of contaminated soil with salicylate as a potential inducer of PAH degradation genes has been explored. But the results have been mixed (Song et al. 2015; Carmichael and Pfaender 1997) and are difficult to interpret: a lack of a stimulation could reflect the fact that salicylate is not an effector for the PAH operons at a site, or that salicylate is consumed by non-PAH degraders. Conversely, if stimulation is observed, it's unclear if that is attributable to induction of PAH degradation genes or to activation of other unknown enzymes.

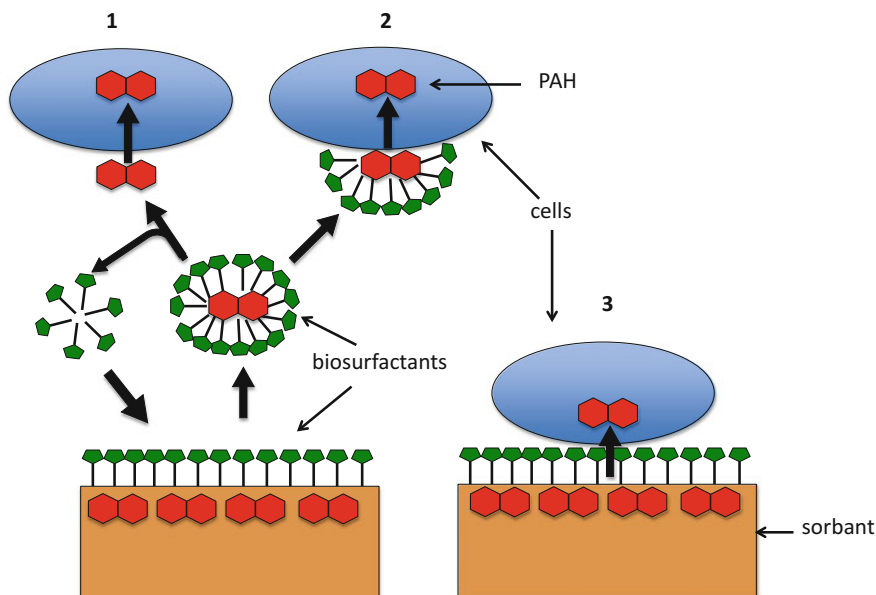
## 7.4 Cellular Uptake of PAH

The key physicochemical characteristic of PAH affecting uptake by bacteria is their strong hydrophobicity, which increases significantly with PAH size, and is reflected in low aqueous solubility and high octanol-water partition coefficients ( $K_{ow}$ , Fig. 7.6). Thus, in the environment, uptake is limited by strong partitioning of PAH to sorbents, primarily natural organic matter and non-aqueous phase liquids (NAPL) of which PAH are constituents. Sorbed PAH are typically regarded as non-bioavailable and inaccessible for uptake, with the transition to a bioavailable state dependent upon relatively slow rates of PAH desorption (Johnsen and Karlson 2004). Thus, while characteristics of enzymes and regulatory elements that mediate catabolism are important, these systems are of little use to the cell if the substrate cannot be acquired. Thus, bioavailability is the single greatest factor limiting bacterial degradation of PAH in the environment (Johnsen et al. 2005; Pouli and Agathos 2011; Juhasz et al. 2014; Mahanty et al. 2011; Rojo-Nieto and Perales-Vargas-Machuca 2012; Simpanen et al. 2016; Yang et al. 2009) and cellular characteristics that may enhance access to sorbed PAH would be crucial to effective degradation of these compounds.

Three pathways have been proposed by which PAH are acquired by bacteria (Fig. 7.7; (Guerin and Boyd 1992; Schippers et al. 2000; Alexander 1994): (1) uptake from aqueous solution, (2) direct uptake from micelles composed of exocellular amphiphiles and (3) direct uptake from sorbents. A wide variety of cellular characteristics and activities can be involved in any of these pathways, but biosurfactant production has a potential role in all three. Biosurfactants are small, amphiphilic molecules possessing hydrophilic and hydrophobic domains (Banat 1995; Lawniczak et al. 2013). There are two main groups of bacterial biosurfactants, glycolipids and peptidolipids (e.g., rhamnolipids and surfactins, respectively), and of these the former have been most extensively studied (Banat 1995; Lawniczak et al. 2013). The term “surfactant” describes the surface-active behavior of these molecules, which preferentially adsorb at interfaces: air-water, liquid-liquid (oil-water) or liquid-solid. The latter two activities are potentially important for PAH uptake as sorption at oil-water boundaries reduces the liquid-liquid interfacial

**Fig. 7.6** Illustration of the relation between hydrophobicity ( $K_{ow}$ ) and molecular weight for the 16 priority pollutant PAH. Values below the horizontal bars indicate the number of rings in the compounds plotted





**Fig. 7.7** Conceptual pathways of PAH uptake by bacteria and potential roles of biosurfactants. *Pathway 1* represents the conventional pathway with uptake occurring from aqueous solution. In this pathway, biosurfactants potentially enhance mass transfer or extraction of the PAH, but there is no direct transfer from the biosurfactant micelle to the bacterial cell; uptake occurs from the PAH dissolved in the aqueous phase. *Pathway 2* depicts direct uptake of PAH sorbed by a biosurfactant micelle. *Pathway 3* shows direct uptake of PAH from a sorbent

tension, while sorption at the liquid-solid interface affects the substrate “wettability”; both of these activities can facilitate cellular interactions with substrates containing PAH. If levels of surfactant secretion are sufficiently high, surfactant monomers aggregate in solution to form colloidal particles called micelles (Pathways 1 and 2) and the surfactant concentration at which that occurs is termed the critical micelle concentration.

Pathways 1 and 2 are similar in that interfacial activities of biosurfactants enhance mass transfer of PAH from a sorbent to the micellar phase, thereby increasing the apparent aqueous solubility of PAH via pseudo-solubilization or emulsification. However, in Pathway 1, uptake is dependent upon the release of PAH from micelles to the aqueous phase, from which bacterial uptake occurs. In contrast, Pathways 2 and 3 illustrate direct uptake, wherein PAH passes directly from the micelle or sorbent into the cell. Direct uptake has been indicated in cases where mineralization rates of PAH or other hydrophobic organic compounds surpass rates that would be predicted if uptake was dependent upon desorption to the aqueous phase (Calvillo and Alexander 1996; Crocker et al. 1995; Efroymson and Alexander 1991; Guerin and Boyd 1997; Harms and Zehnder 1995; Lahlou and Ortega-Calvo 1999; Laor et al. 1999; Tang et al. 1998). Direct uptake has been



indicated for cultures inoculated into soil microcosms, or grown in liquid media containing synthetic surfactants, or humic substances (Guerin and Boyd 1992; Vacca et al. 2005; Guha and Jaffe 1996; Guha et al. 1998). Cellular characteristics that may enable direct uptake are ill defined. But humic acids can interact with cellular membranes (Elayan et al. 2008; Ojwang and Cook 2013), and some bacteria may be able to alter membrane structure in a manner that facilitates direct transfer of PAH to cells when they interact with humic substances containing sorbed PAH (Vacca et al. 2005).

Uptake of PAH via Pathway 3 may have the greatest environmental relevance, as attachment and growth on surfaces as biofilms is the normal state for most bacteria (Dunne 2002; Hall-Stoodley et al. 2004). The surfaces could be solids, such as soil organomineral particles to which PAH sorb, or NAPL that contain PAH, like oil. Direct uptake from these materials is predicated upon adhesion of the cell to the substrate, which is a complex process affected by characteristics of the cell surfaces, the substrate and other physicochemical aspects of environment, like pH and organic and inorganic solutes (Hermansson 1999). Solute are important in forming a “conditioning layer” on the substrate surface (Hermansson 1999), which may modify its physicochemical parameters in a manner that enables a cell surface to reach close proximity and minimize the effective length of diffusion for a sorbed PAH to reach the intracellular catabolic enzymes (Johnsen et al. 2005). Cell surfaces may also become coated with solutes that affect interaction with the solid sorbents or NAPL. While a variety of organic and inorganic molecules can contribute to the conditioning layer and cellular coatings (Hermansson 1999) biosurfactants are potentially of particular importance because of their surface activity (Feng et al. 2013; Liu et al. 2014; Sotirova et al. 2009).

While biosurfactant secretion could facilitate PAH uptake by any of the three pathways, this activity may not be specifically induced by growth on PAH (Chrzanowski et al. 2012). Production of biosurfactants by bacteria growing on PAH has been demonstrated for some cultures, such as fluorescent pseudomonads growing on naphthalene (Deziel et al. 1996; Dasari et al. 2014). However, other investigators have screened a range of Proteobacteria and Actinobacteria grown on a variety of PAH and found little evidence of significant biosurfactant secretion (Johnsen and Karlson 2004; Willumsen and Karlson 1997). Absence of a strong correlation between biosurfactant production and PAH metabolism probably reflects the fact that global regulatory processes controls the former rather than variations in physicochemical characteristics of the carbon source supporting growth (e.g., aqueous solubility (Antonioni et al. 2015)). For example, rhamnolipids have been the most extensively studied class of bacterial biosurfactants, and their biosynthesis is regulated primarily via quorum-sensing systems (Perfumo et al. 2013; Reis et al. 2011). Quorum sensing regulation is particularly important in biofilms, such as those that may be formed by PAH degraders on the surfaces of NAPL or solid sorbants. For example, in the PAH degrader, *Pseudomonas aeruginosa* N6P6, PAH degradation was enhanced by biofilm formation, which in turn was positively correlated expression of *lasI* (Mangwani et al. 2015). The latter is a gene involved in synthesis of quorum sensing signaling molecules, acyl

homoserine lactones. Conversely, inhibiting production of acyl homoserine lactones by *P. aeruginosa* N6P6 diminished both biofilm development and PAH degradation (Mangwani et al. 2015). Thus, quorum sensing can enhance PAH degradation via the promotion of biofilm development, possibly reflecting the beneficial effects of exopolymeric substance (EPS, see below) production and/or biosurfactant secretion. There is as yet no evidence that quorum sensing signaling molecules directly regulate expression of genes encoding enzymes mediating PAH metabolism.

Movement of PAH from a sorbent to the cell cytoplasm necessitates the PAH transverse extra- and intra-cellular regions that differ widely in physico-chemical characteristics (Fig. 7.2). Of particular importance are differences in hydrophobicity/hydrophilicity of these regions, which affect the partitioning behavior of the PAH. As noted above, biofilm formation is likely the natural growth mode for PAH degraders, and a defining characteristic of these structures is the envelopment of cells in a matrix of EPS (Fig. 7.3). Polysaccharides are an essential component of the matrix and provide a foundation for EPS (Bazaka et al. 2011; Fazli et al. 2014; Vu et al. 2009). But, EPS also contain a range of other molecules including lipids and proteins (Dohnalkova et al. 2011; Flemming and Wingender 2010). While the EPS matrix is highly hydrated (>95% water), its heterogeneous composition gives rise to hydrophobic environments on a micro- or nano-scale that may be favorable for entry of PAH, and diffusion to the cell surface (Hobley et al. 2015; Payne and Boles 2016; Zhang et al. 2015).

For most bacteria, the primary cellular boundary is an outer membrane (Sutcliffe 2010; Devos 2014; Sutcliffe et al. 2010), the permeability of which to hydrophobic compounds varies across taxa. In the Proteobacteria, the OM is a barrier for uptake of PAH, a characteristic endowed by the relatively long, hydrophilic carbohydrate chain of the lipopolysaccharide (LPS) dominating the OM outer leaflet. The presence of LPS significantly affects the hydrophobic thickness of the OM, which is ca. 22 Å, compared to ca. 28 Å of the phospholipid bilayer comprising the cytoplasmic membrane (Wu et al. 2014). As noted above, secreted biosurfactants may accumulate on the cell surface offsetting the hydrophilic influence of LPS (Mohanty and Mukherji 2012; Kaczorek et al. 2013; Abbasnezhad et al. 2011), and thereby establish domains conducive to partitioning of hydrophobic compounds. Movement of PAH into and across the OM could thus occur non-specifically, and perhaps be enhanced by biosurfactant modification of cell surface properties (Liu et al. 2014; Li and Zhu 2014).

Transport of PAH across the OM could also be mediated by specific integral OM proteins. The OmpW family of OM proteins possesses hydrophobic channels (Hong et al. 2006; Touw et al. 2010) and has been implicated as important in naphthalene uptake (Neher and Lueking 2009). Another group of potential OM transporters for PAH are FadL-like proteins, which facilitate uptake of hydrophobic compounds by translocation either directly to the periplasm or to the hydrophobic lumen of the OM (Hearn et al. 2008, 2009; van den Berg 2005; van den Berg et al. 2015). Transport by FadL-like proteins is not energy dependent, but instead is driven by conformational changes in the protein following substrate binding

(van den Berg 2005). For hydrocarbons, FadL-like transporters have been best characterized with monoaromatic compounds (e.g., toluene) and include the proteins TodX and TbuX (Kahng et al. 2000; Wang et al. 1995). A FadL-like OM protein had also been associated with uptake of polychlorophenols (Belchik et al. 2010). Whether or not FadL-like proteins have a role in transport of PAH is currently unknown, and as PAH are significantly larger than the monoaromatics, it's possible that the size of PAH may restrict interactions with these transporters.

Two groups of bacteria with OM structure divergent from that of most Proteobacteria are the Sphingomonads of the Alphaproteobacteria (genera *Novosphingobium*, *Sphingobium*, *Sphingopyxis*, and *Sphingomonas*) and *Corynebacterineae* of the *Actinobacteria* (genera *Corynebacterium*, *Mycobacterium*, *Nocardia* and *Rhodococcus*). The OM of Sphingomonads lacks LPS, and instead contains a unique class of glycolipids, glycosphingolipids, which have a carbohydrate chain smaller and less complex than that of LPS, making the OM surface of sphingomonads less hydrophilic than OM containing LPS (Kawahara et al. 1999). The reduced hydrophilicity of the Sphingomonad OM may enhance access to PAH and facilitate interaction with hydrophobic surfaces (Waigi et al. 2015; Regonne et al. 2013). In the *Corynebacterineae*, the OM is often referred to as the mycomembrane (Bansal-Mutalik and Nikaido 2014; Bayan et al. 2003; Zuber et al. 2008), and a defining feature are mycolic acids, which are a class of  $\beta$ -hydroxy fatty acids unique to the *Corynebacterineae*. *Mycolic acids* have long carbon chains, ranging from 30 to 90 carbons in length, which makes the mycomembrane characteristically highly hydrophobic (Marrakchi et al. 2014) and growth on PAH can induce a shift in mycolic acid composition that further enhances hydrophobicity of the mycomembrane (Wick et al. 2002). Consequently, the mycomembrane is a structure that may facilitate acquisition of hydrophobic compounds from the environment, and has been cited as factor contributing to the frequent isolation of genera *Mycobacterium*, *Nocardia* and *Rhodococcus* as *degraders of PAH, especially HMW PAH* (Song et al. 2011; Uyttebroek et al. 2006; Kanaly and Harayama 2010; Kweon et al. 2011; Schneider et al. 1996), and to the association of these genera with hydrophobic sorbents (Regonne et al. 2013; Bastiaens et al. 2000).

The cytoplasmic membrane is the final barrier for compounds entering cells, and for lipophilic chemicals like PAH, passage through this zone can occur primarily by simple diffusion (Gu et al. 2016). This diffusion process is described by the Overton rule (Missner and Pohl 2009), which holds that the permeation of solutes through lipid membranes is proportional to their oil-water partitioning ( $K_{ow}$ ). Thus for PAH, permeation decreases with increasing aqueous solubility (e.g., benzo[*a*]anthracene > fluoranthrene, pyrene > anthracene, phenanthrene). Partitioning of PAH into the cytoplasmic membrane is beneficial as subsequent diffusion to cytoplasm ultimately supplies the cell with a carbon and energy source. However, growth benefits of PAH are counterbalanced by potential toxicity, as PAH partitioning into the lipid bilayer can cause structural deformations that degrade membrane integrity and function (Sikkema et al. 1995). The levels to which PAH need to accumulate in cytoplasmic membranes to inflict toxicity are not well defined, and vary as a

function of the membrane composition, the type of PAH and other environmental variables (Sikkema et al. 1995). Nevertheless, in model membrane systems, structural alterations can occur when PAH levels reach 10 mol% (Korchowiec et al. 2008) and are generally fluidizing effects directly correlated with PAH size (Liland et al. 2014).

The primary cellular response to mitigate deleterious effects of PAH permeation on the cytoplasmic membrane structure is to modify bilayer fluidity. Fluidity is affected by many factors, but a fundamental aspect is lipid packing, which is determined by structural variations in the fatty acyl chain and head group composition of the constituent lipids (Sikkema et al. 1995; Denich et al. 2003; Murinova and Dercova 2014). While systematic studies of alterations in membrane chemistry that accompany bacterial utilization of PAH are lacking, comparative analysis of phospholipid fatty acid (PLFA) composition in cells grown on PAH versus hydrophilic substrates (sugars, complex media) have demonstrated that growth on PAH induces shifts in PLFA composition that are predicted to result in increased membrane fluidity. Two of the most common PLFA alterations that follow PAH exposure are an increased abundance of odd number cyclopropyl fatty acids and decreased ratios of iso- to anteiso-fatty acids (Vacca et al. 2005; Nam et al. 2002; Wick et al. 2003; Kallimanis et al. 2007). An increase in bilayer fluidity may seem counterintuitive as an adaptation for growth on PAH as it would render the cytoplasmic membrane less permeable to these compounds. However, the shift in fluidity could be a strategy to modulate PAH partitioning into the membrane that balances the benefits of PAH uptake that lead to carbon assimilation versus the drawbacks of membrane damage. The extent to which membrane modification is induced in bacteria exposed to PAH in contaminated environments is largely unknown, but there is some evidence that would be consistent with its occurrence. For example, in soil microcosms spiked with PAH, total PLFA decreased with increasing PAH but the fraction (mol%) of cyclopropyl fatty acid increased (Su and Yang 2009). Also, in a creosote-contaminated soil, cyclopropyl fatty acids were among the most abundant PLFA in locations with the highest PAH levels (Bengtsson et al. 2010).

Uptake of PAH into the cytoplasm likely occurs primarily via passive diffusion, but active transport has also been implicated for some strains. In these cases, energy dependent uptake of PAH (naphthalene or phenanthrene) has been reported for *Mycobacterium* (Miyata et al. 2004), *Rhodococcus* sp. BAP-1, *Arthrobacter* (Kallimanis et al. 2007; Li et al. 2014) and *Pseudomonas* (Whitman et al. 1998). Also, for *Sphingomonas paucimobilis* EPA505, specific transport systems have hypothesized based upon mechanistic interpretations of quantitative structure--activity relationship models, which indicated the rate-limiting step in PAH degradation was a binding and transport process, not interaction of the PAH with the PAH-RHD (Dimitriou-Christidis et al. 2008). Active uptake systems have been identified for other hydrophobic compounds, and include an ABC transporter for hexachlorohexane in *Sphingobium japonicum* UT26 (Endo et al. 2007) and an ATP-dependent permease for styrene in *Pseudomonas putida* CA-3 (Mooney et al.

2006). Similar active transport systems for PAH could be present in cases where energy dependent uptake has been indicated, but these have yet to be identified.

A final point to consider is the possibility that bacteria may enhance access to PAH by sensing these compounds and utilize chemotaxis to locate, and move towards, sources of PAH. Chemotactic responses to naphthalene have been reported for several *Pseudomonas* species (Grimm and Harwood 1997; Ortega-Calvo et al. 2003) and in one of these, *Pseudomonas putida* G3, a gene essential for naphthalene chemotaxis was identified (Grimm and Harwood 1999). This gene was located within the operon encoding naphthalene degradation (*nahY*) and the predicted product was a methyl-accepting chemotaxis protein. While the studies with *Pseudomonas* strains demonstrated the potential for chemotaxis, the significance of chemotaxis to PAH biodegradation in the environment is uncertain. A key issue is the constraints on motility, especially in soil. In this regard, soils represent a partially hydrated environment, and water films in a moist soil (field capacity) are estimated to be only on the order of 10 nm thick, which is only a fraction of a cell's diameter and insufficient to support motility (Ebrahimi and Or 2015; Tecon and Or 2016). The roughness of mineral surfaces is an added constraint (Ebrahimi and Or 2015; Tecon and Or 2016). Models considering hydration level and surface roughness indicate that motility is supported only in narrow window of moisture conditions (Ebrahimi and Or 2015; Tecon and Or 2016).

#### **Biomarkers of PAH degradation and their application in microbial ecology.**

As PAH-degrading bacteria present a means to mitigate ecotoxicity of PAH, understanding the environmental activities of these organisms is important, and biomarkers of PAH biodegradation and/or PAH degrading bacteria are needed to do so. From the preceding discussion, it's clear that a myriad of cellular systems impact the ability of bacteria to degrade PAH. But, of these, the only genotypic characteristics that are specifically and uniquely affiliated with PAH biodegradation are genes encoding upper pathway enzymes. The PAH-RHD that initiate upper pathway metabolism has been studied in greatest detail and, as noted above, the PAH-RHD $\alpha$  has received particular focus in the development of PAH-RHD families. Thus, genes encoding PAH-RHD $\alpha$  have been targeted as biomarkers for the analysis of PAH-degraders in the environment, primarily via Polymerase Chain Reaction (PCR) methods. As the PAH-RHD $\alpha$  are phylogenetically diverse, genes encoding these polypeptides exhibit relatively low similarity in nucleotide sequence, and degenerate primers are needed in PCR analyses of PAH-RHD $\alpha$  genes to encompass as much as possible of the PAH-degrader community. Consequently, many PCR primer sets have been developed that in principle enable amplification of different segments of the PAH-RHD $\alpha$  family (Cebron et al. 2008; Bordenave et al. 2008).

Ecological investigations employing PAH-RHD $\alpha$  analyses have yielded insights into the distribution of different PAH-RHD $\alpha$  phylotypes in a variety of environments. These studies have provided information about relationships between PAH-degrader community structure and environmental variables that may affect community structure (Bordenave et al. 2008; Chen et al. 2016; Ding et al. 2010; Flocco et al. 2009; Yang et al. 2014, 2015; Fuentes et al. 2015; Gomes et al. 2007;

Li et al. 2015; Sauret et al. 2016; Zhou et al. 2006). PAH-RHD $\alpha$  analyses has also been employed to assess relationships between PAH-degrader abundance and the PAH levels, and these investigations have yielded mixed results. For example, significant positive correlations of PAH-RHD $\alpha$  gene abundance with PAH levels have been reported for coke factory soils (Han et al. 2014), creosote-contaminated soil (Bengtsson et al. 2013), coal tar-contaminated sediments (Dionisi et al. 2004) and roadside soils (Li et al. 2015). Conversely, a lack of correlation between PAH-RHD $\alpha$  gene abundance and PAH concentrations was demonstrated in roadside soils (Johnsen et al. 2014), oil field soils (Yang et al. 2014) and soils from a former coking plant (Cebron et al. 2009). Reasons for the varying results are unknown, and could include technical issues (e.g., PCR bias in RHD $\alpha$  gene amplification, variations in PAH extraction and analysis, etc.) as well as many physicochemical factors that affect bacterial growth in the environment. For example, heterotrophic bacteria that degrade PAH will also grow on a wide range of other organic compounds that may be present, which could alter PAH-RHD $\alpha$  gene abundance irrespective of PAH concentration. There may also be inhibitory or synergistic metabolic interactions between different PAH that uncouple changes in PAH-RHD $\alpha$  gene abundance from changes in PAH concentration (Hennessee and Li 2016). Also, PAH degraders can utilize only bioavailable PAH, which is a small, variable fraction of the total PAH present in soil or sediment. Thus, differences in total PAH may not be ideal to explain differences in PAH-RHD $\alpha$  gene abundance. For example, in a study of Yangtze river water and sediment, PAH-RHD $\alpha$  gene abundance was positively correlated to levels of dissolved PAH (i.e., bioavailable PAH) in water overlying sediments or in sediment pore water (Xia et al. 2015). However, PAH-RHD $\alpha$  gene abundance was not correlated to total PAH levels in sediment (Xia et al. 2015). Thus, while assessments of PAH-RHD $\alpha$  abundance can be informative about community structure, their use as indicators of PAH biodegradation potential is limited by unknowns about the environmental biology of PAH-degraders, as well as by physicochemical complexities of contaminated soils or sediments containing PAH.

## 7.5 Summary and Future Directions

After several decades of research, much has been learned about the genetic adaptations and processes underlying bacterial metabolism of PAH. The knowledge base is particularly strong in the area of catabolism and characteristics of key enzymes, especially PAH-RHD, which have proven to be a diverse group of proteins. The regulatory systems that control expression of these enzymes have been revealed to be at least equally diverse. Yet, despite the importance of regulation, comparatively little is known about mechanisms by which these systems operate and is a knowledge area in need of significant expansion. Uptake from the environment is the single most important factor affecting PAH degradation and while cellular characteristics that affect uptake are known, the process is still largely a “black box”

and there are few mechanistic details, especially regarding molecules that may facilitate PAH access. Elucidating the mechanisms of uptake is another key research need. Lastly, genes encoding PAH-RHD $\alpha$  have served as useful biomarkers to analyze PAH degrader communities in the environment. Yet, interpreting variations in abundance or distribution of different PAH degrader genotypes has been limited, in part by an incomplete understanding of differences between genotypes in other dimensions of cell biology that affect PAH metabolism (i.e., PAH uptake, gene regulation). Filling these knowledge gaps could help improve the insights that can be gained in ecological studies of PAH biodegradation.

**Acknowledgements** This work was supported by the O.N. Allen Professorship in Soil Microbiology (to WJH). The authors thank Natalie Kirk for assistance in the development of illustrations.

## References

- Abbasnezhad H, Foght JM, Gray MR (2011) Adhesion to the hydrocarbon phase increases phenanthrene degradation by *Pseudomonas fluorescens* LP6a. *Biodegradation* 22(3):485–496
- Albaiges J (2013) Organic chemicals in the environment. In: *Mechanisms of degradation and transformation*, 2nd edn. *Int J Environ Anal Chem* 93(14):1563
- Alekshun MN, Levy SB (1999) The mar regulon: multiple resistance to antibiotics and other toxic chemicals. *Trends Microbiol* 7(10):410–413
- Alexander M (1994) *Biodegradation and bioremediation*. Academic, San Diego, CA
- Antoniou E, Fodelianakis S, Korkakaki E, Kalogerakis N (2015) Biosurfactant production from marine hydrocarbon-degrading consortia and pure bacterial strains using crude oil as carbon source. *Front Microbiol* 6:274
- Baboshin M, Ivashina T, Chernykh A, Golovleva L (2014) Comparison of the substrate specificity of two ring-hydroxylating dioxygenases from *Sphingomonas* sp. VKM B-2434 to polycyclic aromatic hydrocarbons. *Biodegradation* 25(5):693–703
- Banat IM (1995) Biosurfactants production and possible uses in microbial enhanced oil-recovery and oil pollution remediation—a review. *Biores Technol* 51(1):1–12
- Bansal-Mutalik R, Nikaido H (2014) Mycobacterial outer membrane is a lipid bilayer and the inner membrane is unusually rich in diacyl phosphatidylinositol dimannosides. *Proc Natl Acad Sci U S A* 111(13):4958–4963
- Bastiaens L, Springael D, Wattiau P, Harms H, deWachter R, Verachtert H et al (2000) Isolation of adherent polycyclic aromatic hydrocarbon (PAH)-degrading bacteria using PAH-sorbing carriers. *Appl Environ Microbiol* 66(5):1834–1843
- Bayan N, Houssin C, Chami M, Leblon G (2003) Mycomembrane and S-layer: two important structures of *Corynebacterium glutamicum* cell envelope with promising biotechnology applications. *J Biotechnol* 104(1–3):55–67
- Bazaka K, Crawford RJ, Nazarenko EL, Ivanova EP (2011) Bacterial extracellular polysaccharides. In: Linke D, Goldman A (eds) *Bacterial adhesion: chemistry, biology and physics*. *Adv Exp Med Biol* 715:213–226
- Belchik SM, Schaeffer SM, Hasenoehrl S, Xun LY (2010) A beta-barrel outer membrane protein facilitates cellular uptake of polychlorophenols in *Cupriavidus necator*. *Biodegradation* 21(3):431–439



- Bengtsson G, Torneman N, Yang X (2010) Spatial uncoupling of biodegradation, soil respiration, and PAH concentration in a creosote contaminated soil. *Environ Pollut* 158(9):2865–2871
- Bengtsson G, Torneman N, De Liphay JR, Sorensen SJ (2013) Microbial diversity and PAH catabolic genes tracking spatial heterogeneity of PAH concentrations. *Microb Ecol* 65(1):91–100
- Birak PS, Miller CT (2009) Dense non-aqueous phase liquids at former manufactured gas plants: challenges to modeling and remediation. *J Contam Hydrol* 105(3–4):81–98
- Boonchan S, Britz ML, Stanley GA (2000) Degradation and mineralization of high-molecular-weight polycyclic aromatic hydrocarbons by defined fungal-bacterial cocultures. *Appl Environ Microbiol* 66(3):1007–1019
- Bordenave S, Goni-urriza M, Vilette C, Blanchard S, Caumette P, Duran R (2008) Diversity of ring-hydroxylating dioxygenases in pristine and oil contaminated microbial mats at genomic and transcriptomic levels. *Environ Microbiol* 10(12):3201–3211
- Bouchez-Naitali M, Blanchet D, Haeseler F, Vandecasteele JP, Vandecasteele JP (2008) Biodegradation of polycyclic aromatic hydrocarbons (PAHs) 341–411 pp
- Brown DG, Gupta L, Kim T-H, Moo-Young HK, Coleman AJ (2006) Comparative assessment of coal tars obtained from 10 former manufactured gas plant sites in the eastern United States. *Chemosphere* 65(9):1562–1569
- Calvillo YM, Alexander M (1996) Mechanism of microbial utilization of biphenyl sorbed to polyacrylic beads. *Appl Microbiol Biotechnol* 45(3):383–390
- Cao J, Lai Q, Yuan J, Shao Z (2015) Genomic and metabolic analysis of fluoranthene degradation pathway in *Celeribacter indicus* P73(T). *Sci Rep* 5:7741
- Carmichael LM, Pfaender FK (1997) The effect of inorganic and organic supplements on the microbial degradation of phenanthrene and pyrene in soils. *Biodegradation* 8(1):1–13
- Cebron A, Norini MP, Beguiristain T, Leyval C (2008) Real-Time PCR quantification of PAH-ring hydroxylating dioxygenase (PAH-RHD alpha) genes from Gram positive and Gram negative bacteria in soil and sediment samples. *J Microbiol Methods* 73(2):148–159
- Cebron A, Beguiristain T, Faure P, Norini MP, Masfarau JF, Leyval C (2009) Influence of vegetation on the in situ bacterial community and polycyclic aromatic hydrocarbon (PAH) degraders in aged PAH-contaminated or thermal-desorption-treated soil. *Appl Environ Microbiol* 75(19):6322–6330
- Chen SC, Peng JJ, Duan GL (2016) Enrichment of functional microbes and genes during pyrene degradation in two different soils. *J Soils Sediments* 16(2):417–426
- Choi DH, Kwon YM, Kwon KK, Kim S-J (2015) Complete genome sequence of *Novosphingobium pentaromativorans* US6-1(T). *Stan Genomic Sci* 10:107
- Chrzanowski L, Lawniczak L, Czaczyk K (2012) Why do microorganisms produce rhamnolipids? *World J Microbiol Biotechnol* 28(2):401–419
- Crocker FH, Guerin WF, Boyd SA (1995) Bioavailability of naphthalene sorbed to cationic surfactant-modified smectite clay. *Environ Sci Technol* 29(12):2953–2958
- Dalrymple BP, Swadling Y (1997) Expression of a *Butyrivibrio fibrisolvens* E14 gene (cinB) encoding an enzyme with cinnamoyl ester hydrolase activity is negatively regulated by the product of an adjacent gene (cinR). *Microbiology-Sgm* 143:1203–1210
- Dasari S, Subbaiah KCV, Wudayagiri R, Valluru L (2014) Biosurfactant-mediated biodegradation of polycyclic aromatic hydrocarbons-naphthalene. *Bioremediat J* 18(3):258–265
- Denich TJ, Beaudette LA, Lee H, Trevors JT (2003) Effect of selected environmental and physico-chemical factors on bacterial cytoplasmic membranes. *J Microbiol Methods* 52(2):149–182
- Devos DP (2014) PVC bacteria: variation of, but not exception to, the Gram-negative cell plan. *Trends Microbiol* 22(1):14–20
- Deziel E, Paquette G, Villemur R, Lepine F, Bisailon JG (1996) Biosurfactant production by a soil *Pseudomonas* strain growing on polycyclic aromatic hydrocarbons. *Appl Environ Microbiol* 62(6):1908–1912

- Di Gennaro P, Terreni P, Masi G, Botti S, De Ferra F, Bestetti G (2010) Identification and characterization of genes involved in naphthalene degradation in *Rhodococcus opacus* R7. *Appl Microbiol Biotechnol* 87(1):297–308
- Diaz E, Prieto MA (2000) Bacterial promoters triggering biodegradation of aromatic pollutants. *Curr Opin Biotechnol* 11(5):467–475
- Diaz E, Jimenez JJ, Nogales J (2013) Aerobic degradation of aromatic compounds. *Curr Opin Biotechnol* 24(3):431–442
- Dimitriou-Christidis P, Autenrieh RL, Abraham MH (2008) Quantitative structure-activity relationships for kinetic parameters of polycyclic aromatic hydrocarbon biotransformation. *Environ Toxicol Chem* 27(7):1496–1504
- Ding GC, Heuer H, Zuhlke S, Spiteller M, Pronk GJ, Heister K et al (2010) Soil type-dependent responses to phenanthrene as revealed by determining the diversity and abundance of polycyclic aromatic hydrocarbon ring-hydroxylating dioxygenase genes by using a novel PCR detection system. *Appl Environ Microbiol* 76(14):4765–4771
- Dionisi HM, Chewning CS, Morgan KH, Menn FM, Easter JP, Sayler GS (2004) Abundance of dioxygenase genes similar to *Ralstonia* sp. strain U2 nagAc is correlated with naphthalene concentrations in coal tar-contaminated freshwater sediments. *Appl Environ Microbiol* 70(7):3988–3995
- Dohnalkova AC, Marshall MJ, Arey BW, Williams KH, Buck EC, Fredrickson JK (2011) Imaging hydrated microbial extracellular polymers: comparative analysis by electron microscopy. *Appl Environ Microbiol* 77(4):1254–1262
- Dong C, Bai X, Lai Q, Xie Y, Chen X, Shao Z (2014) Draft genome sequence of *Sphingobium* sp. strain C100, a polycyclic aromatic hydrocarbon-degrading bacterium from the deep-sea sediment of the Arctic Ocean. *Genome Announcements* 2(1):e01210–e01213
- Doyle E, Muckian L, Hickey AM, Clipson N (2008) Microbial PAH degradation. In: Laskin AI, Sariaslani S, Gadd GM (eds) *Advances in applied microbiology*, vol 65. *Advances in applied microbiology*, pp 27–66
- Dunne WM (2002) Bacterial adhesion: seen any good biofilms lately? *Clin Microbiol Rev* 15(2):155–166
- Ebrahimi A, Or D (2015) Hydration and diffusion processes shape microbial community organization and function in model soil aggregates. *Water Resour Res* 51(12):9804–9827
- Efroymsen RA, Alexander M (1991) Biodegradation by an arthrobacter species of hydrocarbons partitioned into an organic-solvent. *Appl Environ Microbiol* 57(5):1441–1447
- Elayan NM, Treleaven WD, Cook RL (2008) Monitoring the effect of three humic acids on a model membrane system using P-31 NMR. *Environ Sci Technol* 42(5):1531–1536
- Endo R, Ohtsubo Y, Tsuda M, Nagata Y (2007) Identification and characterization of genes encoding a putative ABC-type transporter essential for utilization of gamma-hexachlorocyclohexane in *Sphingobium japonicum* UT26. *J Bacteriol* 189(10):3712–3720
- Fazli M, Almlad H, Rytbke ML, Givskov M, Eberl L, Tolker-Nielsen T (2014) Regulation of biofilm formation in *Pseudomonas* and *Burkholderia* species. *Environ Microbiol* 16(7):1961–1981
- Feng WH, Swift S, Singhal N (2013) Effects of surfactants on cell surface tension parameters and hydrophobicity of *Pseudomonas putida* 852 and *Rhodococcus erythropolis* 3586. *Colloids Surf B-Biointerfaces* 105:43–50
- Fernandez-Luqueno F, Valenzuela-Encinas C, Marsch R, Martinez-Suarez C, Vazquez-Nunez E, Dendooven L (2011) Microbial communities to mitigate contamination of PAHs in soil-possibilities and challenges: a review. *Environ Sci Pollut Res* 18(1):12–30
- Flemming HC, Wingender J (2010) The biofilm matrix. *Nat Rev Microbiol* 8(9):623–633
- Flocco CG, Gomes NCM, Mac Cormack W, Smalla K (2009) Occurrence and diversity of naphthalene dioxygenase genes in soil microbial communities from the Maritime Antarctic. *Environ Microbiol* 11(3):700–714

- Fuenmayor SL, Wild M, Boyes AL, Williams PA (1998) A gene cluster encoding steps in conversion of naphthalene to gentisate in *Pseudomonas* sp. strain U2. *J Bacteriol* 180(9): 2522–2530
- Fuentes S, Ding GC, Cardenas F, Smalla K, Seeger M (2015) Assessing environmental drivers of microbial communities in estuarine soils of the Aconcagua River in Central Chile. *Fems Microbiol Ecol* 91(10):flv110
- Gallegos MT, Schleif R, Bairoch A, Hofmann K, Ramos JL (1997) AraC/XylS family of transcriptional regulators. *Microbiol Mol Biol Rev* 61(4):393–410
- Gomes NCM, Borges LR, Paranhos R, Pinto FN, Krogerrecklenfort E, Mendonca-Hagler LCS et al (2007) Diversity of *ndo* genes in mangrove sediments exposed to different sources of polycyclic aromatic hydrocarbon pollution. *Appl Environ Microbiol* 73(22):7392–7399
- Grimm AC, Harwood CS (1997) Chemotaxis of *Pseudomonas* spp. to the polyaromatic hydrocarbon naphthalene. *Appl Environ Microbiol* 63(10):4111–4115
- Grimm AC, Harwood CS (1999) NahY, a catabolic plasmid-encoded receptor required for chemotaxis of *Pseudomonas putida* to the aromatic hydrocarbon naphthalene. *J Bacteriol* 181(10):3310–3316
- Gu HP, Lou J, Wang HZ, Yang Y, Wu LS, Wu JJ et al (2016) Biodegradation, biosorption of phenanthrene and its trans-membrane transport by *Massilia* sp. WF1 and *Phanerochaete chrysosporium*. *Front Microbiol* 7
- Guerin WF, Boyd SA (1992) Differential bioavailability of soil-sorbed naphthalene to 2 bacterial species. *Appl Environ Microbiol* 58(4):1142–1152
- Guerin WF, Boyd SA (1997) Bioavailability of naphthalene associated with natural and synthetic sorbents. *Water Res* 31(6):1504–1512
- Guha S, Jaffe PR (1996) Bioavailability of hydrophobic compounds partitioned into the micellar phase of nonionic surfactants. *Environ Sci Technol* 30(4):1382–1391
- Guha S, Jaffe PR, Peters CA (1998) Bioavailability of mixtures of PAHs partitioned into the micellar phase of a nonionic surfactant. *Environ Sci Technol* 32(15):2317–2324
- Habe H, Omori T (2003) Genetics of polycyclic aromatic hydrocarbon metabolism in diverse aerobic bacteria. *Biosci Biotechnol Biochem* 67(2):225–243
- Hall-Stoodley L, Costerton JW, Stoodley P (2004) Bacterial biofilms: from the natural environment to infectious diseases. *Nat Rev Microbiol* 2(2):95–108
- Han X-M, Liu Y-R, Zheng Y-M, Zhang X-X, He J-Z (2014) Response of bacterial *pdo1*, *nah*, and *C120* genes to aged soil PAH pollution in a coke factory area. *Environ Sci Pollut Res* 21(16):9754–9763
- Harms H, Zehnder AJB (1995) Bioavailability of sorbed 3-chlorodibenzofuran. *Appl Environ Microbiol* 61(1):27–33
- Harvey RG (1997) Polycyclic aromatic hydrocarbons. Wiley-VCH, New York
- Hearn EM, Patel DR, van den Berg B (2008) Outer-membrane transport of aromatic hydrocarbons as a first step in biodegradation. *Proc Natl Acad Sci U S A* 105(25):8601–8606
- Hearn EM, Patel DR, Lepore BW, Indic M, van den Berg B (2009) Transmembrane passage of hydrophobic compounds through a protein channel wall. *Nature* 458(7236):367–370
- Hennessee CT, Li QX (2016) Effects of polycyclic aromatic hydrocarbon mixtures on degradation, gene expression, and metabolite production in four mycobacterium species. *Appl Environ Microbiol* 82(11):3357–3369
- Hermansson M (1999) The DLVO theory in microbial adhesion. *Colloids Surf B-Biointerfaces* 14(1–4):105–119
- Hobley L, Harkins C, MacPhee CE, Stanley-Wall NR (2015) Giving structure to the biofilm matrix: an overview of individual strategies and emerging common themes. *FEMS Microbiol Rev* 39(5):649–669
- Hong HD, Patel DR, Tamm LK, van den Berg B (2006) The outer membrane protein *OmpW* forms an eight-stranded beta-barrel with a hydrophobic channel. *J Biol Chem* 281(11): 7568–7577

- Huang JZ, Schell MA (1991) In vivo interactions of the NahR transcriptional activator with its target sequences—inducer-mediated changes resulting in transcription activation. *J Biol Chem* 266(17):10830–10838
- Jain PK (2015) Microbial biodegradation of polycyclic aromatic hydrocarbons. In: Harzevili FD, Chen H (eds), 331–349 pp
- Johnsen AR, Karlson U (2004) Evaluation of bacterial strategies to promote the bioavailability of polycyclic aromatic hydrocarbons. *Appl Microbiol Biotechnol* 63(4):452–459
- Johnsen AR, Wick LY, Harms H (2005) Principles of microbial PAH-degradation in soil. *Environ Pollut* 133(1):71–84
- Johnsen AR, Styriehave B, Aamand J (2014) Quantification of small-scale variation in the size and composition of phenanthrene-degrader populations and PAH contaminants in traffic-impacted topsoil. *FEMS Microbiol Ecol* 88(1):84–93
- Johnston CG, Johnston GP (2012) Bioremediation of polycyclic aromatic hydrocarbons. In: *Microbial biotechnology: energy and environment*, pp 279–296
- Jones RM, Britt-Compton B, Williams PA (2003) The naphthalene catabolic (nag) genes of *Ralstonia* sp. strain U2 are an operon that is regulated by NagR, a LysR-type transcriptional regulator. *J Bacteriol* 185(19):5847–5853
- Jouanneau Y, Meyer C, Jakoncic J, Stojanoff V, Gaillard J (2006) Characterization of a naphthalene dioxygenase endowed with an exceptionally broad substrate specificity toward polycyclic aromatic hydrocarbons. *Biochemistry* 45(40):12380–12391
- Juhász AL, Aleer S, Adetutu EM (2014) Predicting PAH bioremediation efficacy using bioaccessibility assessment tools: validation of PAH biodegradation-bioaccessibility correlations. *Int Biodeterior Biodegradation* 95:320–329
- Kaczorek E, Salek K, Guzik U, Jesionowski T, Cybulski Z (2013) Biodegradation of alkyl derivatives of aromatic hydrocarbons and cell surface properties of a strain of *Pseudomonas stutzeri*. *Chemosphere* 90(2):471–478
- Kahng HY, Byrne AM, Olsen RH, Kukor JJ (2000) Characterization and role of *tbuX* in utilization of toluene by *Ralstonia pickettii* PKO1. *J Bacteriol* 182(5):1232–1242
- Kallimanis A, Frillingos S, Drainas C, Koukkou AI (2007) Taxonomic identification, phenanthrene uptake activity, and membrane lipid alterations of the PAH degrading *Arthrobacter* sp. strain Sphe3. *Appl Microbiol Biotechnol* 76(3):709–717
- Kallimanis A, Karabika E, Mavromatis K, Lapidus A, LaButti KM, Liolios K et al (2011) Complete genome sequence of *Mycobacterium* sp. strain (Splyr1) and reclassification to *Mycobacterium gilvum* Splyr1. *Stan Genomic Sci* 5(1):144–153
- Kallscheuer N, Vogt M, Kappellmann J, Krumbach K, Noack S, Bott M et al (2016) Identification of the *phd* gene cluster responsible for phenylpropanoid utilization in *Corynebacterium glutamicum*. *Appl Microbiol Biotechnol* 100(4):1871–1881
- Kanally RA, Harayama S (2010) Advances in the field of high-molecular-weight polycyclic aromatic hydrocarbon biodegradation by bacteria. *Microb Biotechnol* 3(2):136–164
- Kasai Y, Shindo K, Harayama S, Misawa N (2003) Molecular characterization and substrate preference of a polycyclic aromatic hydrocarbon dioxygenase from *Cycloclasticus* sp. strain A5. *Appl Environ Microbiol* 69(11):6688–6697
- Kawahara K, Kuraishi H, Zahringer U (1999) Chemical structure and function of glycosphingolipids of *Sphingomonas* spp. and their distribution among members of the alpha-4 subclass of Proteobacteria. *J Ind Microbiol Biotechnol* 23(4–5):408–413
- Khara P, Roy M, Chakraborty J, Ghosal D, Dutta TK (2014) Functional characterization of diverse ring-hydroxylating oxygenases and induction of complex aromatic catabolic gene clusters in *Sphingobium* sp. PNB. *FEBS Open Bio* 4:290–300
- Kim SJ, Kweon O, Freeman JP, Jones RC, Adjei MD, Jhoo JW et al (2006) Molecular cloning and expression of genes encoding a novel dioxygenase involved in low- and high-molecular-weight polycyclic aromatic hydrocarbon degradation in *Mycobacterium vanbaalenii* PYR-1. *Appl Environ Microbiol* 72(2):1045–1054

- Kim SJ, Kweon O, Jones RC, Edmondson RD, Cerniglia CE (2008) Genomic analysis of polycyclic aromatic hydrocarbon degradation in *Mycobacterium vanbaalenii* PYR-1. *Biodegradation* 19(6):859–881
- Kiyohara H, Nagao K, Kouno K, Yano K (1982) Phenanthrene-degrading phenotype of *alcaligenes-faecalis* AFK2. *Appl Environ Microbiol* 43(2):458–461
- Korchowec B, Corvis Y, Viitala T, Feidt C, Guiavarch Y, Corbier C et al (2008) Interfacial approach to polyaromatic hydrocarbon toxicity: phosphoglyceride and cholesterol monolayer response to phenanthrene, anthracene, pyrene, chrysene, and benzo a pyrene. *J Phys Chem B* 112(43):13518–13531
- Krivobok S, Kuony S, Meyer C, Louwagie M, Willison JC, Jouanneau Y (2003) Identification of pyrene-induced proteins in *Mycobacterium* sp. strain 6PY1: evidence for two ring-hydroxylating dioxygenases. *J Bacteriol* 185(13):3828–3841
- Kulakov LA, Chen SC, Allen CCR, Larkin MJ (2005) Web-type evolution of *Rhodococcus* gene clusters associated with utilization of naphthalene. *Appl Environ Microbiol* 71(4):1754–1764
- Kuppusamy S, Thavamani P, Megharaj M, Naidu R (2016) Biodegradation of polycyclic aromatic hydrocarbons (PAHs) by novel bacterial consortia tolerant to diverse physical settings—assessments in liquid- and slurry-phase systems. *Int Biodeterior Biodegradation* 108:149–157
- Kwak Y, Park G-S, Lee S-E, Li QX, Shin J-H (2014) Genome sequence of *Mycobacterium aromaticivorans* JS19b1(T), a novel isolate from Hawaiian soil. *J Biotechnol* 186:137–138
- Kweon O, Kim S-J, Freeman JP, Song J, Baek S, Cerniglia CE (2010) Substrate specificity and structural characteristics of the novel Rieske nonheme iron aromatic ring-hydroxylating oxygenases NidAB and NidA3B3 from *Mycobacterium vanbaalenii* PYR-1. *MBio* 1(2): e00135-10
- Kweon O, Kim S-J, Holland RD, Chen H, Kim D-W, Gao Y et al (2011) Polycyclic aromatic hydrocarbon metabolic network in *Mycobacterium vanbaalenii* PYR-1. *J Bacteriol* 193(17):4326–4337
- Ladino-Orjuela G, Gomes E, da Silva R, Salt C, Parsons JR (2016) Metabolic pathways for degradation of aromatic hydrocarbons by bacteria. In: DeVooigt P (ed) *Reviews of environmental contamination and toxicology*, vol 237. *Rev Environ Contam Toxicol* 237:105–121
- Lafortune I, Juteau P, Deziel E, Lepine F, Beaudet R, Villemur R (2009) Bacterial diversity of a consortium degrading high-molecular-weight polycyclic aromatic hydrocarbons in a two-liquid phase biosystem. *Microb Ecol* 57(3):455–468
- Lahlou M, Ortega-Calvo JJ (1999) Bioavailability of labile and desorption-resistant phenanthrene sorbed to montmorillonite clay containing humic fractions. *Environ Toxicol Chem* 18(12):2729–2735
- Lai Q, Li W, Wang B, Yu Z, Shao Z (2012) Complete genome sequence of the pyrene-degrading bacterium *Cycloclasticus* sp. strain P1. *J Bacteriol* 194(23):6677
- Laor Y, Strom PF, Farmer WJ (1999) Bioavailability of phenanthrene sorbed to mineral-associated humic acid. *Water Res* 33(7):1719–1729
- Larkin MJ, Kulakov LA, Allen CCR (2005) Biodegradation and *Rhodococcus*—masters of catabolic versatility. *Curr Opin Biotechnol* 16(3):282–290
- Laurie AD, Lloyd-Jones G (1999) The *phn* genes of *Burkholderia* sp. strain RP007 constitute a divergent gene cluster for polycyclic aromatic hydrocarbon catabolism. *J Bacteriol* 181(2):531–540
- Lawniczak L, Marecik R, Chrzanowski L (2013) Contributions of biosurfactants to natural or induced bioremediation. *Appl Microbiol Biotechnol* 97(6):2327–2339
- Li F, Zhu L (2014) Surfactant-modified fatty acid composition of *Citrobacter* sp SA01 and its effect on phenanthrene transmembrane transport. *Chemosphere* 107:58–64
- Li Y, Wang H, Hua F, Su M, Zhao Y (2014) Trans-membrane transport of fluoranthene by *Rhodococcus* sp. BAP-1 and optimization of uptake process. *Biores Technol* 155:213–219
- Li XF, Hou LJ, Liu M, Zheng YL, Li Y, Lin XB (2015) Abundance and diversity of polycyclic aromatic hydrocarbon degradation bacteria in urban roadside soils in Shanghai. *Appl Microbiol Biotechnol* 99(8):3639–3649

- Li Z-Y, Wu Y-H, Huo Y-Y, Cheng H, Wang C-S, Xu X-W (2016) Complete genome sequence of a benzo a pyrene-degrading bacterium *Altererythrobacter epoxidivorans* CGMCC 1.7731(T). *Mar Genomics* 25:39–41
- Liland NS, Simonsen AC, Duelund L, Torstensen BE, Berntssen MHG, Mouritsen OG (2014) Polyaromatic hydrocarbons do not disturb liquid-liquid phase coexistence, but increase the fluidity of model membranes. *Chem Phys Lipid* 184:18–24
- Liu Y, Ma X, Zeng G, Zhong H, Liu Z, Jiang Y et al (2014) Role of low-concentration monorhamnolipid in cell surface hydrophobicity of *Pseudomonas aeruginosa*: adsorption or lipopolysaccharide content variation. *Appl Microbiol Biotechnol* 98(24):10231–10241
- Lonneborg R, Brzezinski P (2011) Factors that influence the response of the LysR type transcriptional regulators to aromatic compounds. *BMC Biochemistry* 12(1):49
- Maddocks SE, Oyston PCF (2008) Structure and function of the LysR-type transcriptional regulator (LTTR) family proteins. *Microbiology-Sgm* 154:3609–3623
- Maeda AH, Nishi S, Ozeki Y, Ohta Y, Hatada Y, Kanaly RA (2013) Draft genome sequence of *Sphingobium* sp. strain KK22, a high-molecular-weight polycyclic aromatic hydrocarbon-degrading bacterium isolated from cattle pasture soil. *Genome Announcements* 1(6)
- Mahanty B, Pakshirajan K, Dasu VV (2011) Understanding the complexity and strategic evolution in PAH remediation research. *Crit Rev Environ Sci Technol* 41(19):1697–1746
- Mangwani N, Kumari S, Das S (2015) Involvement of quorum sensing genes in biofilm development and degradation of polycyclic aromatic hydrocarbons by a marine bacterium *Pseudomonas aeruginosa* N6P6. *Appl Microbiol Biotechnol* 99(23):10283–10297
- Marrakchi H, Laneelle M-A, Daffe M (2014) Mycolic acids: structures, biosynthesis, and beyond. *Chem Biol* 21(1):67–85
- Messina E, Denaro R, Crisafi F, Smedile F, Cappello S, Genovese M et al (2016) Genome sequence of obligate marine polycyclic aromatic hydrocarbons-degrading bacterium *Cycloclasticus* sp. 78-ME, isolated from petroleum deposits of the sunken tanker Amoco Milford Haven, Mediterranean Sea. *Mar Genomics* 25:11–13
- Missner A, Pohl P (2009) 110 years of the Meyer-Overton rule: predicting membrane permeability of gases and other small compounds. *ChemPhysChem* 10(9–10):1405–1414
- Miyata N, Iwahori K, Foght JM, Gray MR (2004) Saturable, energy-dependent uptake of phenanthrene in aqueous phase by *Mycobacterium* sp. strain RJGII-135. *Appl Environ Microbiol* 70(1):363–369
- Mohanty S, Mukherji S (2012) Alteration in cell surface properties of *Burkholderia* spp. during surfactant-aided biodegradation of petroleum hydrocarbons. *Appl Microbiol Biotechnol* 94(1):193–204
- Mooney A, O’Leary ND, Dobson ADW (2006) Cloning and functional characterization of the *styE* gene, involved in styrene transport in *Pseudomonas putida* CA-3. *Appl Environ Microbiol* 72(2):1302–1309
- Moser R, Stahl U (2001) Insights into the genetic diversity of initial dioxygenases from PAH-degrading bacteria. *Appl Microbiol Biotechnol* 55(5):609–618
- Murinova S, Dercova K (2014) Response mechanisms of bacterial degraders to environmental contaminants on the level of cell walls and cytoplasmic membrane. *Int J Microbiol* 2014
- Nam K, Moon HS, Kim JY, Kukor JJ (2002) Linkage between biodegradation of polycyclic aromatic hydrocarbons and phospholipid profiles in soil isolates. *J Microbiol Biotechnol* 12(1):77–83
- Neher TM, Lueking DR (2009) *Pseudomonas fluorescens* ompW: plasmid localization and requirement for naphthalene uptake. *Can J Microbiol* 55(5):553–563
- Nojiri H, Habe H, Omori T (2001) Bacterial degradation of aromatic compounds via angular dioxygenation. *J Gen Appl Microbiol* 47(6):279–305
- Ohtsubo Y, Delawary M, Kimbara K, Takagi M, Ohta A, Nagata Y (2001) BphS, a key transcriptional regulator of bph genes involved in polychlorinated biphenyl/biphenyl degradation in *Pseudomonas* sp. KKS102. *J Biol Chem* 276(39):36146–36154

- Ojwang LM, Cook RL (2013) Environmental conditions that influence the ability of humic acids to induce permeability in model biomembranes. *Environ Sci Technol* 47(15):8280–8287
- Ortega-Calvo JJ, Marchenko AI, Vorobyov AV, Borovick RV (2003) Chemotaxis in polycyclic aromatic hydrocarbon-degrading bacteria isolated from coal-tar- and oil-polluted rhizospheres. *FEMS Microbiol Ecol* 44(3):373–381
- Pagnout C, Frache G, Poupin P, Maunit B, Muller J-F, Ferard J-F (2007) Isolation and characterization of a gene cluster involved in PAH degradation in *Mycobacterium* sp. strain SNP11: expression in *Mycobacterium smegmatis* mc(2)155. *Res Microbiol* 158(2):175–186
- Pan T, Huang P, Xiong G, Maser E (2015) Isolation and identification of a repressor TetR for 3,17 beta-HSD expressional regulation in *Comamonas testosteroni*. *Chem Biol Interact* 234:205–212
- Parales RE, Ju KS (2011) Rieske-type dioxygenases: key enzymes in the degradation of aromatic hydrocarbons. In: *Comprehensive biotechnology*, vol 6: Environmental biotechnology and safety, 2nd edn, pp 115–134
- Park W, Padmanabhan P, Padmanabhan S, Zylstra GJ, Madsen EL (2002) nahR, encoding a LysR-type transcriptional regulator, is highly conserved among naphthalene-degrading bacteria isolated from a coal tar waste-contaminated site and in extracted community DNA. *Microbiology-SGM* 148:2319–2329
- Park HH, Lim WK, Shin HJ (2005) In vitro binding of purified NahR regulatory protein with promoter P<sub>sal</sub>. *Biochim Biophys Acta-General Subjects* 1725(2):247–255
- Payne DE, Boles BR (2016) Emerging interactions between matrix components during biofilm development. *Curr Genet* 62(1):137–141
- Peng R-H, Xiong A-S, Xue Y, Fu X-Y, Gao F, Zhao W et al (2008) Microbial biodegradation of polyaromatic hydrocarbons. *FEMS Microbiol Rev* 32(6):927–955
- Peng J-J, Wang N, Li H-J, Cai C (2011) Microbial degradation mechanisms of soil high molecular weight PAHs and affecting factors: a review. *Shengtaixue Zazhi* 30(11):2621–2627
- Perfumo A, Rudden M, Smyth TJP, Marchant R, Stevenson PS, Parry NJ et al (2013) Rhamnolipids are conserved biosurfactants molecules: implications for their biotechnological potential. *Appl Microbiol Biotechnol* 97(16):7297–7306
- Pouli M, Agathos SN (2011) Bioremediation of PAH-contaminated sites: from pathways to bioreactors. In: Koukkou AI (ed), 119–147 pp
- Regonne RK, Martin F, Mbawala A, Ngassoum MB, Jouanneau Y (2013) Identification of soil bacteria able to degrade phenanthrene bound to a hydrophobic sorbent in situ. *Environ Pollut* 180:145–151
- Reis RS, Pereira AG, Neves BC, Freire DMG (2011) Gene regulation of rhamnolipid production in *Pseudomonas aeruginosa*—a review. *Biores Technol* 102(11):6377–6384
- Rojo-Nieto E, Perales-Vargas-Machuca JA (2012) Microbial degradation of PAHs: organisms and environmental compartments. In: Singh SN (ed) *Microbial degradation of xenobiotics*. *Environ Sci* 263–290
- Sauret C, Tedetti M, Guigue C, Dumas C, Lami R, Pujo-Pay M et al (2016) Influence of PAHs among other coastal environmental variables on total and PAH-degrading bacterial communities. *Environ Sci Pollut Res* 23(5):4242–4256
- Schell MA (1985) Transcriptional control of the nah and sal hydrocarbon-degradation operons by the nahR gene-product. *Gene* 36(3):301–309
- Schell MA, Brown PH, Raju S (1990) Use of saturation mutagenesis to localize probable functional domains in the NahR protein, a LysR-type transcription activator. *J Biol Chem* 265(7):3844–3850
- Schippers C, Gessner K, Muller T, Scheper T (2000) Microbial degradation of phenanthrene by addition of a sophorolipid mixture. *J Biotechnol* 83(3):189–198
- Schneider J, Grosser R, Jayasimhulu K, Xue W, Warshawsky D (1996) Degradation of pyrene, benz a anthracene, and benzo a pyrene by *Mycobacterium* sp. strain RJGII-135, isolated from a former coal gasification site. *Appl Environ Microbiol* 62(1):13–19



- Schuler L, Chadhain SMN, Jouanneau Y, Meyer C, Zylstra GJ, Hols P et al (2008) Characterization of a novel angular dioxygenase from fluorene-degrading *Spingomonas* sp. strain LB126. *Appl Environ Microbiol* 74(4):1050–1057
- Seo J-S, Keum Y-S, Li QX (2009) Bacterial degradation of aromatic compounds. *Int J Environ Res Public Health* 6(1):278–309
- Seo J-S, Keum Y-S, Li QX (2012) *Mycobacterium aromativorans* JS19b1(T) degrades phenanthrene through C-1,2, C-3,4 and C-9,10 dioxygenation pathways. *Int Biodeterior Biodegradation* 70:96–103
- Shetty AR, de Gannes V, Obi CC, Lucas S, Lapidus A, Cheng JF et al (2015) Complete genome sequence of the phenanthrene-degrading soil bacterium *Delftia acidovorans* Cs 1-4. *Stand Genomic Sci* 10
- Sikkema J, Debont JAM, Poolman B (1995) Mechanisms of membrane toxicity of hydrocarbons. *Microbiol Rev* 59(2):201–222
- Simpanen S, Makela R, Mikola J, Silvennoinen H, Romantschuk M (2016) Bioremediation of creosote contaminated soil in both laboratory and field scale: Investigating the ability of methyl-beta-cyclodextrin to enhance biostimulation. *Int Biodeterior Biodegradation* 106:117–126
- Song X, Xu Y, Li G, Zhang Y, Huang T, Hu Z (2011) Isolation, characterization of *Rhodococcus* sp P14 capable of degrading high-molecular-weight polycyclic aromatic hydrocarbons and aliphatic hydrocarbons. *Mar Pollut Bull* 62(10):2122–2128
- Song M, Luo CL, Jiang LF, Zhang DY, Wang YJ, Zhang G (2015) Identification of benzo a pyrene-metabolizing bacteria in forest soils by using DNA-based stable-isotope probing. *Appl Environ Microbiol* 81(21):7368–7376
- Sotirova A, Spasova D, Vasileva-Tonkova E, Galabova D (2009) Effects of rhamnolipid-biosurfactant on cell surface of *Pseudomonas aeruginosa*. *Microbiol Res* 164 (3):297–303
- Stach JEM, Burns RG (2002) Enrichment versus biofilm culture: a functional and phylogenetic comparison of polycyclic aromatic hydrocarbon-degrading microbial communities. *Environ Microbiol* 4(3):169–182
- Story SP, Parker SH, Hayasaka SS, Riley MB, Kline EL (2001) Convergent and divergent points in catabolic pathways involved in utilization of fluoranthene, naphthalene, anthracene, and phenanthrene by *Sphingomonas paucimobilis* var. EPA505. *J Ind Microbiol Biotechnol* 26(6):369–382
- Su YH, Yang XY (2009) Interactions between selected PAHs and the microbial community in rhizosphere of a paddy soil. *Sci Total Environ* 407(3):1027–1034
- Sutcliffe IC (2010) A phylum level perspective on bacterial cell envelope architecture. *Trends Microbiol* 18(10):464–470
- Sutcliffe LC, Brown AK, Dover LG (2010) The rhodococcal cell envelope: composition, organisation and biosynthesis. In: Alvarez HM (ed) *Biology of Rhodococcus*. Microbiology monographs, vol 16, pp 29–71
- Tang WC, White JC, Alexander M (1998) Utilization of sorbed compounds by microorganisms specifically isolated for that purpose. *Appl Microbiol Biotechnol* 49(1):117–121
- Tang H, Yu H, Li Q, Wang X, Gai Z, Yin G et al (2011) Genome sequence of *Pseudomonas putida* strain B6-2, a superdegrader of polycyclic aromatic hydrocarbons and dioxin-like compounds. *J Bacteriol* 193(23):6789–6790
- Tecon R, Or D (2016) Bacterial flagellar motility on hydrated rough surfaces controlled by aqueous film thickness and connectedness. *Sci Rep* 6:19409
- Touw DS, Patel DR, van den Berg B (2010) The crystal structure of OprG from *Pseudomonas aeruginosa*, a potential channel for transport of hydrophobic molecules across the outer membrane. *PLoS ONE* 5(11)
- Tropel D, van der Meer JR (2004) Bacterial transcriptional regulators for degradation pathways of aromatic compounds. *Microbiol Mol Biol Rev* 68(3):474–500

- Uyttebroek M, Ortega-Calvo J-J, Breugelmans P, Springael D (2006) Comparison of mineralization of solid-sorbed phenanthrene by polycyclic aromatic hydrocarbon (PAH)-degrading *Mycobacterium* spp. and *Sphingomonas* spp. *Appl Microbiol Biotechnol* 72(4):829–836
- Vacca DJ, Bleam WF, Hickey WJ (2005) Isolation of soil bacteria adapted to degrade humic acid-sorbed phenanthrene. *Appl Environ Microbiol* 71(7):3797–3805
- Vaidehi K, Kulkarni SD (2012) Microbial remediation of polycyclic aromatic hydrocarbons: an overview. *Res J Chem Environ* 16(4):200–212
- Vamsee-Krishna C, Phale PS (2008) Bacterial degradation of phthalate isomers and their esters. *Indian J Microbiol* 48(1):19–34
- van den Berg B (2005) The FadL family: unusual transporters for unusual substrates. *Curr Opin Struct Biol* 15(4):401–407
- van den Berg B, Bhamidimarri SP, Winterhalter M (2015) Crystal structure of a COG4313 outer membrane channel. *Sci Rep* 5
- Van Hamme JD, Singh A, Ward OP (2003) Recent advances in petroleum microbiology. *Microbiol Mol Biol Rev* 67(4):503–549
- Vinas M, Sabate J, Guasp C, Lalucat J, Solanas AM (2005) Culture-dependent and -independent approaches establish the complexity of a PAH-degrading microbial consortium. *Can J Microbiol* 51(11):897–909
- Vu B, Chen M, Crawford RJ, Ivanova EP (2009) Bacterial Extracellular polysaccharides involved in biofilm formation. *Molecules* 14(7):2535–2554
- Waigi MG, Kang F, Goikavi C, Ling W, Gao Y (2015) Phenanthrene biodegradation by sphingomonads and its application in the contaminated soils and sediments: a review. *Int Biodeterior Biodegradation* 104:333–349
- Wang Y, Rawlings M, Gibson DT, Labbe D, Bergeron H, Brousseau R et al (1995) Identification of a membrane-protein and a truncated LysR-type regulator associated with the toluene degradation pathway in *Pseudomonas putida* F1. *Mol Gen Genet* 246(5):570–579
- Wang X, Jin D, Zhou L, Wu L, An W, Zhao L (2014a) Draft genome sequence of *Advenella kashmirensis* strain W13003, a polycyclic aromatic hydrocarbon-degrading bacterium. *Genome Announcements* 2(1):e00003–e00014
- Wang X, Jin D, Zhou L, Wu L, Qi L, Li C et al (2014b) Draft genome sequence of halotolerant polycyclic aromatic hydrocarbon-degrading *Pseudomonas bauzanensis* strain W13Z2. *Genome Announcements* 2(5):e01049–e01114
- Wang X, Jin D, Zhou L, Zhang Z (2015) Draft genome sequence of *Ochrobactrum anthropi* strain W13P3, a halotolerant polycyclic aromatic hydrocarbon-degrading bacterium. *Genome Announcements* 3(4):e00867–e00915
- Wang X, Jin D, Zhou L, Zhang Z (2016) Draft genome sequence of *Pannonibacter phragmitetus* strain CGMCC9175, a halotolerant polycyclic aromatic hydrocarbon-degrading bacterium. *Genome Announcements* 4(1):e01675–e01715
- Wehrer M, Rennert T, Mansfeldt T, Totsche KU (2011) Contaminants at former manufactured gas plants: sources, properties, and processes. *Crit Rev Environ Sci Technol* 41(21):1883–1969
- Whitman BE, Lueking DR, Mihelcic JR (1998) Naphthalene uptake by a *Pseudomonas fluorescens* isolate. *Can J Microbiol* 44(11):1086–1093
- Wick LY, Wattiau P, Harms H (2002) Influence of the growth substrate on the mycolic acid profiles of mycobacteria. *Environ Microbiol* 4(10):612–616
- Wick LY, Pelz O, Bernasconi SM, Andersen N, Harms H (2003) Influence of the growth substrate on ester-linked phospho- and glycolipid fatty acids of PAH-degrading *Mycobacterium* sp. LB501T. *Environ Microbiol* 5(8):672–680
- Wilkinson SP, Grove A (2006) Ligand-responsive transcriptional regulation by members of the MarR family of winged helix proteins. *Curr Issues Mol Biol* 8:51–62
- Willumsen PA, Karlson U (1997) Screening of bacteria, isolated from PAH-contaminated soils, for production of biosurfactants and bioemulsifiers. *Biodegradation* 7(5):415–423
- Wu EL, Fleming PJ, Yeom MS, Widmalm G, Klauda JB, Fleming KG et al (2014) *E. coli* outer membrane and interactions with OmpLA. *Biophys J* 106(11):2493–2502

- Xia XH, Xia N, Lai YJ, Dong JW, Zhao PJ, Zhu BT et al (2015) Response of PAH-degrading genes to PAH bioavailability in the overlying water, suspended sediment, and deposited sediment of the Yangtze River. *Chemosphere* 128:236–244
- Yang YJ, Chen RF, Shiaris MP (1994) Metabolism of naphthalene, fluorene, and phenanthrene - preliminary characterization of a cloned gene-cluster from *Pseudomonas-putida* NCIB-9816. *J Bacteriol* 176(8):2158–2164
- Yang Y, Hunter W, Tao S, Gan J (2009) Microbial availability of different forms of phenanthrene in soils. *Environ Sci Technol* 43(6):1852–1857
- Yang YY, Wang J, Liao JQ, Xie SG, Huang Y (2014) Distribution of naphthalene dioxygenase genes in crude oil-contaminated soils. *Microb Ecol* 68(4):785–793
- Yang Y, Wang J, Liao J, Xie S, Huang Y (2015) Abundance and diversity of soil petroleum hydrocarbon-degrading microbial communities in oil exploring areas. *Appl Microbiol Biotechnol* 99(4):1935–1946
- Yen KM, Serdar CM (1988) Genetics of naphthalene catabolism in pseudomonads. *CRC Crit Rev Microbiol* 15(3):247–268
- Zhang Y, Qin F, Qiao J, Li G, Shen C, Huang T et al (2012) Draft genome sequence of *Rhodococcus* sp. strain P14, a biodegrader of high-molecular-weight polycyclic aromatic hydrocarbons. *J Bacteriol* 194(13):3546–3546
- Zhang W, Sun J, Ding W, Lin J, Tan R, Lu L et al (2015) Extracellular matrix-associated proteins form an integral and dynamic system during *Pseudomonas aeruginosa* biofilm development. *Front Cell Infect Microbiol* 5
- Zhou HW, Guo CL, Wong YS, Tam Nfy (2006) Genetic diversity of dioxygenase genes in polycyclic aromatic hydrocarbon-degrading bacteria isolated from mangrove sediments. *FEMS Microbiol Lett* 262(2):148–157
- Zuber B, Chami M, Houssin C, Dubochet J, Griffiths G, Daffe M (2008) Direct visualization of the outer membrane of mycobacteria and corynebacteria in their native state. *J Bacteriol* 190 (16):5672–5680