Polycyclic aromatic hydrocarbons: environmental pollution and bioremediation

Sudip K. Samanta, Om V. Singh and Rakesh K. Jain

Polycyclic aromatic hydrocarbons (PAHs) are widely distributed and relocated in the environment as a result of the incomplete combustion of organic matter. Many PAHs and their epoxides are highly toxic, mutagenic and/or carcinogenic to microorganisms as well as to higher systems including humans. Although various physicochemical methods have been used to remove these compounds from our environment, they have many limitations. Xenobiotic-degrading microorganisms have tremendous potential for bioremediation but new modifications are required to make such microorganisms effective and efficient in removing these compounds, which were once thought to be recalcitrant. Metabolic engineering might help to improve the efficiency of degradation of toxic compounds by microorganisms. However, efficiency of naturally occurring microorganisms for field bioremediation could be significantly improved by optimizing certain factors such as bioavailability, adsorption and mass transfer. Chemotaxis could also have an important role in enhancing biodegradation of pollutants. Here, we discuss the problems of PAH pollution and PAH degradation, and relevant bioremediation efforts.

Published online: 9 April 2002

Sudip K. Samanta
Om V. Singh
Rakesh K. Jain*
Institute of Microbial Technology, Sector-39A, Chandigarh-160036, India.
*e-mail: rkj@imtech.res.in

A wide variety of polycyclic aromatic hydrocarbons (PAHs) (Fig. 1) are found in the environment as a result of the incomplete combustion of organic matter, emission sources, automobile exhausts, stationary matter (e.g. coal-fired, electricity-generating power plants), domestic matter (e.g. tobacco smoke and residential wood or coal combustion), area source matter (e.g. forest fires and agricultural burning) and also in food [1]. Massive relocation of natural materials to different areas of the ecosystem has taken place during the past several decades as a result of human activity, thus exposing living systems to these different compounds [2]. Some PAHs (e.g. naphthalene and phenanthrene) have also been used in the synthesis of different organic compounds in pesticides, fungicides, detergents, dyes and mothballs [3].

Toxicity

Many PAHs have toxic, mutagenic and/or carcinogenic properties [4,5]. PAHs are highly lipid-soluble and thus readily absorbed from the gastro-intestinal tract of mammals [6]. They are rapidly distributed in a wide variety of tissues with a marked tendency for localization in body fat. Metabolism of PAHs occurs via the cytochrome P450-mediated mixed function oxidase system with oxidation or hydroxylation as the first step [7]. The resultant epoxides or phenols might get detoxified in a reaction to produce glucoronides, sulfates or glutathione conjugates. Some of the epoxides might metabolize into dihydrodiols, which in turn, could undergo conjugation to form soluble detoxification products or be oxidized to diol-epoxides. Many PAHs contain a 'bay-region' as well as 'K-region', both of which allow metabolic formation of bay- and K-region epoxides, which are highly reactive. Carcinogenicity has been demonstrated by some of these epoxides [4] (Fig. 2). Therefore, many PAHs are considered to be environmental pollutants that can have a detrimental effect on the flora and fauna of affected habitats, resulting in the uptake and accumulation of toxic chemicals in food chains and, in some instances, in serious health problems and/or genetic defects in humans. Consequently, the US Environmental Protection Agency has listed 16 PAHs as priority pollutants for remediation [8].

Naphthalene, the first member of the PAH group, is a common micropollutant in potable water. The toxicity of naphthalene has been well documented and cataractogenic activity has been reported in laboratory animals [4,5]. Naphthalene binds covalently to molecules in liver, kidney and lung tissues, thereby enhancing its toxicity; it is also an inhibitor of mitochondrial respiration [9]. Acute naphthalene poisoning in humans can lead to haemolytic anaemia and nephrotoxicity. In addition, dermal and ophthalmological changes have been observed in workers occupationally exposed to naphthalene. Phenanthrene is known to be a photosensitizer of human skin, a mild allergen and mutagenic to bacterial systems under specific conditions [5]. It is a weak inducer of sister chromatid exchanges and a potent inhibitor of gap junctional intercellular communication [10]. Equivocal results for tumour initiation have been obtained with skin-painting studies in mice. Interestingly, because phenanthrene is the smallest PAH to have a bay-region and a K-region, it is often used as a model substrate for studies on the metabolism of carcinogenic PAHs [11]. Little information is available for other PAHs such as acenaphthene, fluranthene and flourene with respect to their toxicity in mammals. However, the toxicity of benzo(a)pyrene, benzo(a)anthracene, benzo(b)fluoranthene,

  PII: S0167-7799(02)01943-1

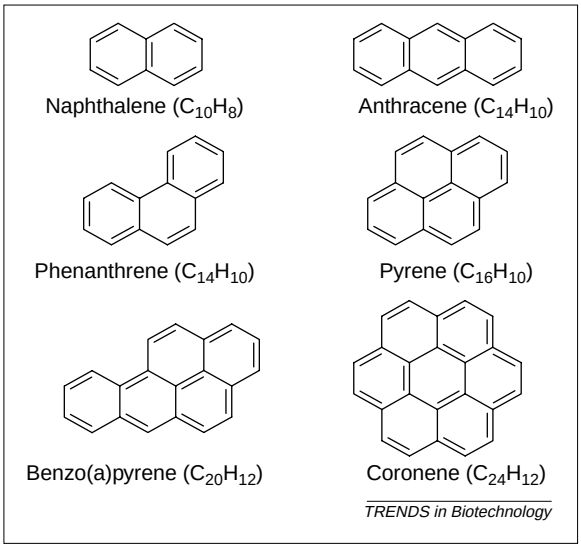

Fig. 1. Structure of some abundant polycyclic aromatic hydrocarbons (PAHs) in the environment. Such PAHs range from simple naphthalene to complex coronene and are the most abundant organic molecules in space, making up to 20% of the total cosmic carbon.

benzo(k)fluranthene, dibenz(a,h)anthracene and indeno(1,2,3-c,d)pyrene has been studied and there is sufficient experimental evidence to show that they are carcinogenic [5,8,12]. PAHs are rarely encountered alone in the environment and many interactions occur within a mixture of PAHs whereby the potency of known genotoxic and carcinogenic PAHs can be enhanced [13]. For example, 1-nitropyrene, a nitrated PAH, is produced during reactions between ketones in products of burning automobile fuel and airborne nitrogen oxides that take place on the surface of hydrocarbon particles in diesel exhaust. In the Ames *Salmonella typhimurium* assay, 1-nitropyrene was found to be highly mutagenic and carcinogenic, whereas the parent compound, pyrene, is non-carcinogenic and only weakly mutagenic [14].

Microbial degradation

The first step in the microbial degradation of PAHs is the action of dioxygenase, which incorporates atoms of oxygen at two carbon atoms of a benzene ring of a PAH resulting in the formation of *cis*-dihydrodiol [15], which undergoes rearomatization by dehydrogenases to form dihydroxylated intermediates. Dihydroxylated intermediates subsequently undergo ring cleavage and form TCA-cycle intermediates [16]. A large number of naphthalene-degrading microorganisms (including *Alcaligenes denitrificans*, *Mycobacterium* sp., *Pseudomonas putida*, *P. fluorescens*, *P. paucimobilis*, *P. vesicularis*, *P. cepacia*, *P. testosteroni*, *Rhodococcus* sp., *Corynebacterium venale*, *Bacillus cereus*, *Moraxella* sp., *Streptomyces* sp., *Vibrio* sp. and *Cyclotrophicus* sp.) has been isolated and examined for mineralization [17,18]. In 1976, Kiyohara and coworkers reported the isolation of a phenanthrene-degrading bacterium [19]. Subsequently, there have been many reports of phenanthrene degradation by different bacteria including *Aeromonas* sp., *Alcaligenes faecalis*, *A. denitrificans*, *Arthrobacter polychromogenes*, *Beijerinckia* sp., *Micrococcus* sp., *Mycobacterium* sp., *Pseudomonas putida*, *P. paucimobilis*, *Rhodococcus* sp., *Vibrio* sp., *Nocardia* sp., *Flavobacterium* sp., *Streptomyces* sp. and *Bacillus* sp. [20,21].

In the past decade, research into the bacterial biodegradation of PAHs composed of more than three rings has advanced significantly. Of the four-ring PAHs, biodegradation of fluoranthene, pyrene, chrysene and benz[a]anthracene has been investigated to various degrees [15,22]. *Stenotrophomonas maltophilia* strain VUN 10 003 was evaluated in a basal liquid medium for fluoranthene degradation and co-metabolization of other PAHs, including pyrene, benz[a]anthracene and coronene [22]. Fluoranthene metabolites resulting from degradation by a *Mycobacterium* sp. have been reported and >95% fluoranthene can be degraded if efficient organic nutrients are provided in a mineral medium. Several actinomycetes bacteria, such as *Mycobacterium* sp., *Gardona* sp. and *Rhodococcus* sp., were isolated from varying hydrocarbon-contaminated soils and each uses fluoranthene, pyrene and chrysene as sole carbon and energy sources [23]. A variety of non-actinomycete bacteria, such as *P. putida*, *P. aeruginosa*, *P. fluorescence*, *Burkholderia cepacia*, *S. yanoikuyae*, *Flavobacterium* sp. and *Cycloclasticus* sp., has been investigated to see if these can metabolize benz[a]anthracene, chrysene, fluoranthene and pyrene [15,24]. A few studies have also documented the bacterial degradation of PAHs with five or more rings in both environmental samples and pure or mixed cultures. However, most research has been targeted towards benzo[a]pyrene (BaP), a five-ring molecule, abundantly present as an active component of coal tar. Although BaP has been detected in a variety of environmental samples [15], so far, no microorganism has been reported that can use BaP as a sole source of carbon and energy. A slight degradation of BaP in a six-component PAHs mixture has been reported with *Mycobacterium* sp. [25], and *S. paucimobilis* can degrade the five ring PAH dibenz[a,b]anthracene and benzo[b]fluoranthene from 7.5% to 33% [26].

During the past decade, a variety of microorganisms has been isolated and characterized for the ability to degrade different PAHs, and new pathways for PAH degradation have been elucidated. Further research is needed to explore the microbial interactions within PAH-degrading consortia, the regulatory mechanisms of various ring-structured PAH biodegradation as well as the co-metabolic biodegradation of PAHs. The new approach of advancements in molecular biology can aid in the detection of PAH-degrading organisms from environmental samples [24,27]. DNA–DNA hybridization has been directly applied to detect and monitor the crucial populations recovered from the environment [28,29]. Laurie and Jones [30] detected two distinct PAH catabolic genotypes from aromatic hydrocarbon-contaminated soil using quantitative

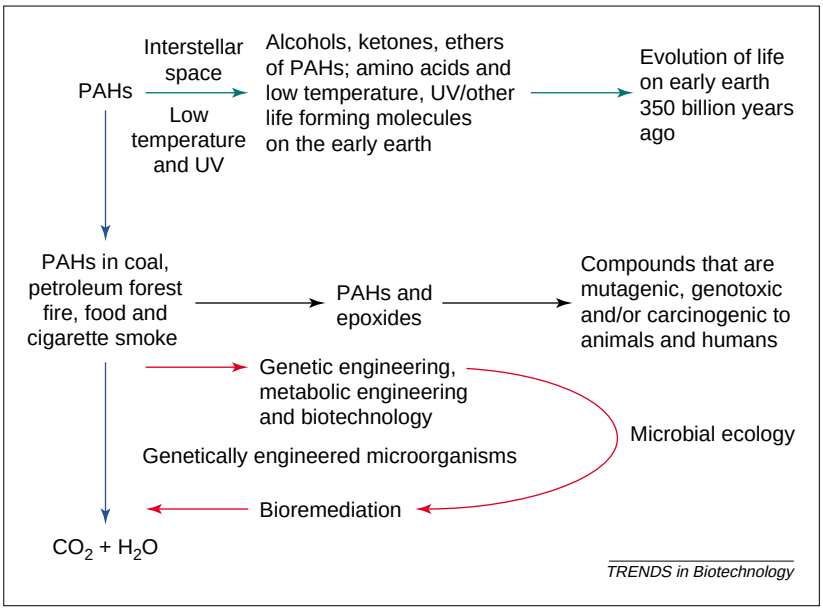

Fig. 2. Fate, toxicity and remediation of polycyclic aromatic hydrocarbons (PAHs) from the environment. A wide variety of PAHs are abundant in nature owing to incomplete combustion of organic matters. The PAHs from extraterrestrial matter are also oxidized and reduced owing to prevalent astrophysical conditions and resulting in the formation of various organic molecules, which are the basis of early life on primitive earth. The microorganisms (naturally occurring or genetically engineered) can mineralize toxic PAHs into CO_2 and H_2O.

competitive PCR (QC-PCR). A soil-derived consortium capable of rapidly mineralizing benzo[a]pyrene was analyzed using denaturing gradient gel electrophoresis (DGGE) profiling of PCR-amplified 16S rDNA fragments [31]. This analysis detected 16S rDNA sequence types that represented organisms closely related to known high molecular weight PAH-degrading bacteria. Recently, many PAH-degrading bacteria have been isolated from geographically diverse sites using the 16S rRNA sequence technique [32].

Chemotaxis

To enhance the degradation of toxic compounds in the environment, different strategies can be considered with chemotaxis as one of the potential means to achieve this goal. From the biodegradation aspect in natural environment, microorganisms that have degradation capability and also show chemotaxis towards a compound would be more efficient for bioremediation than non-chemotactic microorganisms. Although chemotaxis is a phenomenon that has been known for some time (and there are several reports in the literature regarding chemotaxis of *E. coli*, *Salmonella*, *P. aeruginosa*, *P. putida*, *Bacillus cereus*, *Myxococcus* sp., *Rhizobium* sp. and *Azospirillum* sp.) [33,34], it is a complex process in which bacterial cells detect temporal changes in the concentrations of specific chemicals, respond behaviourally to these changes and then adapt to the new concentration of the chemical stimuli. Chemotaxis can be positive (i.e. the microorganism migrate towards the compound) or negative (i.e. microorganisms swim away from the compound). Both cases require concentration gradients of the attractant or repellent for a chemotactic response to occur. Some toxic organic compounds are chemoattractant for different bacterial species, such as *Pseudomonas* sp. and *Ralstonia* sp. [35,36], which could lead to improved degradation.

The chemotaxis of *Pseudomonas putida* towards naphthalene and salicylate is a plasmid-encoded phenomenon, encoded by the catabolic plasmid NAH7 [37] residing within this strain. A chemotaxis gene region has been identified from *P. putida* that was found to be homologous to chemotaxis, flagellar and mobility genes from some other bacteria [38]. It has also been shown that a 5.9 kb *Eco*RI fragment encoding a chemoreceptor Nah Y adjacent to the catabolic gene in NAH7 is involved in naphthalene chemotaxis [35]. Another naphthalene- and salicylate-degrading plasmid, pRKJ1, has also been reported [39]. The transfer of pRKJ1 into plasmid-free *P. putida* KT2442 resulted in the acquisition of chemotaxis and degradation properties. The recombinant plasmid pRKJ3 (containing 25 kb *Eco*RI fragment in pLAFR3) was transferred into the plasmid-free strain of RKJ1, RKJ5, and was shown to be chemotactic towards both naphthalene and salicylate (Table 1). However, strains KT2442 or RKJ5 containing only the vector pLAFR3 did not show any chemotaxis towards naphthalene and salicylate. This established the role of the 25 kb *Eco*RI fragment in chemotaxis associated with complete mineralization of these compounds [39]. These results imply that chemotaxis towards naphthalene and/or salicylate might be owing to changes in cellular energy (redox) levels, which provide signals to bacteria for chemotactic behaviour. Alternatively, it might be because of intracellular chemoreceptors that recognize such contaminants or their degradative product(s), which subsequently provide chemotactic signals to the organism. This energy-dependent chemotactic response is a widely accepted phenomenon in other microbes such as *E. coli* and *Rhodobacter sphaeroides* [40,41]. In our laboratory, work is in progress to establish whether this metabolism alone is sufficient for chemotaxis, or whether plasmid-encoded chemoreceptor is involved in this physiological response by separately subcloning catabolic along with the regulatory and potential chemoreceptor gene(s). Further work is required to establish whether a metabolism of the substrates or binding of the substrates to chemoreceptor(s) is the crucial criterion for chemotaxis. Understandably, the chemotaxis phenomenon is expected to receive considerable attention with respect to bioremediation.

Genetically engineered microorganisms (GEMs)

The adverse environmental conditions might not permit the survival of an efficient natural degrader

Table 1. Chemotaxis response of *Pseudomonas putida* RKJ1 and plasmid towards naphthalene and salicylate

Strain of *Pseudomonas putida*	Drop assay		Swarm plate assay		Capillary assay (CI)[a]	
	Nap.	Sal.	Nap.	Sal.	Nap.	Sal.
RKJ1	+++[b]	++	+++	++	2.60	5.10
KT2442/pRKJ1	+++	+	+++	+	ND	ND
RKJ5/pRKJ3	+++	+	+++	+	ND	ND
KT2442/pLAFR3	0	0	0	0	ND	ND

[a]Reported as the average of the CI values obtained from four sets of experiments using duplicate capillaries.
[b]Response rates from 0 (no response) to +++ (strong response), measured both as diameter and degree of contrast.
Abbreviations: Nap., napthalene; ND, not determined; Sal., salicylate.
(This table has been adapted from [39].

in a natural ecosystem, consequently, it might show less efficiency in comparison with laboratory conditions. It is possible that by enhancing the enzymatic activity of biochemical pathway(s) using genetic engineering (resulting in higher expression of key enzymes), improved degradation of many persistent compounds that are abundant in the environment could be achieved. Various biotechnological techniques might not only be useful for the development of efficient microorganisms for environmental remediation [42,43] (Fig. 2) but also such organisms might be useful for specific remediation of environmental pollutants. Although there are scanty reports on the use of GEMs in bioremediation, they are a promising candidate for bioremediation. Several genetic engineering tools such as gene conversion, gene duplication, transposition and bio-vehicles (plasmids and transposons) might have vital roles in the rearrangement of DNA fragments as well as in the activation or inactivation of cryptic genes and to facilitate crossover of the significant genetic information from host cells to recipients [42].

To some extent, molecular biology approaches have been used to evaluate bioremediation and assess the elimination of toxicity from the contaminated sites [44,45]. The characterization of degradative plasmids helps in the study of the genetics of catabolism and the ability to monitor expression of specific catabolic genes is of immense importance in evaluating the feasibility of *in situ* bioremediation strategies. Enhanced rates of PAH mineralization have been shown, together with a corresponding increase in the number of colonies containing DNA sequences that hybridize TOL and NAH plasmid probes using colony hybridization [28]. Specific inducers of genetic operons can also be used to enhance the degradation of toxic compounds. Salicylate is one such inducer of both the upper and lower operons carried on NAH7 plasmid for degradation of naphthalene and salicylate, respectively [46]. The role, as well as the specificity, of salicylate as an inducer of operons involved in the biodegradation of PAH has been reported [47]. The genetic characterization of *phn* genes of *Burkholderia* sp. strain RP007 led to a significant understanding of the process of bacterial PAH degradation [48]. Recently, the dioxygenase gene(s) was successfully cloned, subcloned and overexpressed in *E. coli* with the pBAD Thio Fusion system from *Mycobacterium* sp. strain PYR-1 responsible for higher molecular weight PAH degradation [49].

Genetic engineering has also provided an adequate strategy to develop GEMs that are able to sense an environmental contaminant and respond to it through easily detectable signals such as bioluminescence [50]. Such GEMs could be useful in reporting the availability and the biodegradation of PAHs via illuminating signals that could be used as online tool for *in situ* monitoring of bioremediation processes. Metabolic engineering and sequence diversity in genes for bioremediation of aromatic compounds is not only broad but also new genes and genetic organizations are still being discovered [51]. Scientists are only just beginning to discover the broad flexibility of microorganisms with the help of newly established methods, for example new genes, enzymatic and metabolic routes for better exploitation of natural diversity for PAH degradation and bioremediation [49–52].

Microorganisms in field studies

Over the past decade, there has been increased research into bioremediation of PAHs and related compounds in the environment. As discussed, PAHs can be degraded by a variety of naturally occurring soil bacteria, which have proved to be effective at the field scale [52–54]. Indigenous populations of PAH- and phenol-utilizing microorganisms were used to remediate the soil from a creosote plant containing PAHs and phenols using an *ex situ* land treatment process [53]. This showed 82–97% degradation of lower PAHs (and up to 35% degradation of higher PAHs) and biostimulation was also found to be effective in the process [53]. Various chemical conditions and mass transfer effects were analyzed under conditions of *in situ* bioremediation of a coal tar contaminated aquifer at the site of a shut down manufactured gas plant to assess the efficacy of degradation by indigenous naturally occurring microorganisms [55]. Bioremediation of experimental oil spills on the shoreline on Delaware Bay was carried out with three kinds of oil samples [56]; these findings provided a basis for a framework under which petroleum-based hydrocarbon-containing soils could be evaluated from the ecological perspective. Bioremediation using naturally occurring microorganisms was a major mechanism of removing oil from the *Exxon Valdez* oil spill in Prince William Sound, Alaska [57]. Furthermore, various environmental factors

(biotic and abiotic) have been shown to influence the ability of microbial populations to mineralize polycyclic aromatic and aliphatic compounds [58]. The implications for nutrient-amended bioremediation were studied in a hydrocarbon-contaminated Arctic soil. The greatest stimulation in microbial activity occurred at the lowest, rather than the highest, level of nutrient addition [59]. An indigenously selected natural bacterial consortium of five bacterial strains has been applied at sites containing petroleum hydrocarbon at a refinery in India, and the inoculum levels of the survival rates of introduced microorganisms were evaluated [60]. Bioremediation of diesel-oil contaminated soil in an Alpine Glacier skiing area has been carried out in fertilized and unfertilized soil over three successive summer sessons [61]. In microcosm studies, PAH degradation was successfully achieved by natural microorganisms [62,63]. Actual oil-contaminated Kuwaiti soil was used for remediation of recalcitrant PAHs in soil microcosms showing 20–45% degradation [62]. Three types of soil matrices were used to trace the mineralization, transformation and extractability of PAHs in microcosms [63]. The mineralization of PAHs in soil is usually slow because the bioavailability is limited by a poor mass transfer owing to strong or irreversible sorption, besides certain biotic and abiotic factors that also have a vital role in biodegradation [52,58,60]. However, as discussed, this has not prevented successful field-scale bioremediation programmes. The limited success rates might be because some sites are heavily contaminated, and microorganisms are unable to grow as soon as they are inoculated. The bioremediation processes can be more effective with suitable environmental conditions for growth of microorganisms.

The application of GEMs might be useful for treatment of heavily contaminated sites for bioremediation purposes. Genetic modification achieved through cloning of genes of biodegradation pathway(s) with broader substrate specificities could enhance biodegradation rates. However, it is essential to check the stability of any GEMs before its field application [64]. The fate of released GEM depends largely on the stability of the recombinant plasmid present in an organism. A bioluminescent strain was used on a field site to estimate the survival and dispersion of a GEM [65]. The field release of GEM *Pseudomonas fluorescens* HK44 for the purpose of bioremediation was used [66] and the benefits and obstacles associated with the use of GEMs in bioremediation applications have been reviewed [42–44].

GEMs that are introduced into the environment might be able to move in a variety of environment media (air, soil and water) and multiply when conditions become favourable. Questions regarding the effects of an introduced microorganism arise whenever the intended introduction differs substantially from those with an established record of safety. The concept of releasing GEMs into the environment requires a clear understanding of their behaviour, dispersal, survival and the ability to detect and monitor the fate of genes and organisms within a microbial community. Therefore, exploitation of GEMs is likely to be restricted both *in situ* and *ex situ* bioremediation in the near future. An alternative assessment to release the GEMs in the environment can be proposed on the basis of familiarity with organisms, their genetic modification, the ability to confine the organism and the perceived environmental impact.

Conclusions

Since the origin of the earth, environmental contamination can be viewed as an ecological malaise and bioremediation can be prescribed as 'environmental medicine'. Also, after the post-contamination treatment, bioremediation could be used as a preventive medicine for the future. Bioremediation is a multidisciplinary treatment technique with the central thrust pertaining to microbiological perspectives and it is essential to know the natural habitat of the degradative microbial populations before stating cost effective, ecologically safe and environmentally sound bioremediation plans. Total field bioremediation is often a difficult task whether using GEMs or intrinsic microorganisms. Primarily, such problems lie in finding out up to what extent the microbes themselves are contributing to the degradation process and in the recognition of different related factors occurring within the degradation system such as chemical transformation and volatilization.

Acknowledgements
We are grateful to Amit Ghosh, our Director, for his encouragement. This is IMTECH Communication No. 023/2001.

References

1 Finlayson-Pitts, B.J. and Pitts, J.N., Jr (1997) Tropospheric air pollution: ozone, airborne toxics, polycyclic aromatic hydrocarbons and particles. *Science* 276, 1045–1052
2 Fiala, Z. *et al.* (1999) Polycyclic aromatic hydrocarbons I. Environmental contamination and environmental exposure. *Acta Medica* 42, 77–89
3 Shennan, J.L. (1984) Hydrocarbons as substrates in industrial fermentation. In *Petroleum Microbiology* (Atlas, R.M., ed), pp. 643–683, Macmillan
4 Goldman, R. *et al.* (2001) Smoking increase carcinogenic polycyclic aromatic hydrocarbons in human lung tissue. *Cancer Res.* 61, 6367–6371
5 Mastrangela, G. *et al.* (1997) Polycyclic aromatic hydrocarbons and cancer in man. *Environ. Health Perspect.* 104, 1166–1170
6 Cerniglia, C.E. (1984) Microbial metabolism of polycyclic aromatic hydrocarbons. *Adv. Appl. Microbiol.* 30, 31–71
7 Stegeman, J.J. *et al.* (2001) Cytochrome P450 1A expression in mid water fishes: potential effects of chemical contaminants in remote oceanic zones. *Environ. Sci. Technol.* 35, 54–62
8 Liu, K. *et al.* (2001) Polycyclic aromatic hydrocarbon (PAH) emissions from a coal fired pilot FBC system. *J. Hazard. Mater.* 84, 175–188
9 Falahatpisheh, M.H. *et al.* (2001) Antagonistic interactions among nephrotoxic polycyclic aromatic hydrocarbons. *J. Toxicol. Environ. Health* 62, 543–560
10 Weis, L.M. *et al.* (1998) Bay and baylike regions of polycyclic aromatic hydrocarbons were potent inhibitors of gap junctional intercellular communication. *Environ. Health Perspect.* 106, 17–22

11 Bucker, M. *et al.* (1979) Mutagenicity of phenanthrene and phenanthrene K-region derivatives. *Mutat. Res.* 66, 337–348
12 Sram, R.J. *et al.* (1999) Adverse reproductive outcomes from exposure to environmental mutagens. *Mutat. Res.* 428, 203–215
13 Kaiser, J. (1997) Endocrine disrupters: Synergy paper questioned at toxicology meeting. *Science* 275, 1879–1880
14 Pothuluri, J.V. and Cerniglia, C.E. (1994) Microbial metabolism of polycyclic aromatic hydrocarbons. In *Biological degradation and Bioremediation of Toxic Chemicals* (Chaudhry, G.R., ed.), pp. 92–124, Chapman and Hall
15 Kanaly, R.A. and Harayama, S. (2000) Biodegradation of high-molecular-weight polycyclic aromatic hydrocarbons by bacteria. *J. Bacteriol.* 182, 2059–2067
16 Sabate, J. *et al.* (1999) Isolation and characterization of a 2-methylphenanthrene utilizing bacterium: identification of ring cleavage metabolites. *Appl. Microbiol. Biotechnol.* 52, 704–712
17 Hedlund, B.P. and James, T.S. (2001) *Vibrio cyclotrophicus* sp. nov., a polycyclic aromatic hydrocarbon (PAH)-degrading marine bacterium. *Int. J. Sys. Evol. Microbiol.* 51, 61–66
18 Samanta, S.K. *et al.* (2001) Efficiency of naphthalene and salicylate degradation by a recombinant *Pseudomonas putida* mutant strain defective in glucose metabolism. *Appl. Microbiol. Biotechnol.* 55, 627–631
19 Kiyohara, H. *et al.* (1976) Degradation of phenanthrene through *o*-phthalate by a *Aeromonas* sp. *Agr. Biol. Chem.* 40, 1075–1082
20 Samanta, S.K. *et al.* (1999) Degradation of phenanthrene by different bacteria: evidence for novel transformation sequences involving the formation of 1-naphthol. *Appl. Microbiol. Biotechnol.* 53, 98–107
21 Johri, A.K. *et al.* (1999) Characterization and regulation of catabolic genes. *Crit. Rev. Microbiol.* 25, 245–273
22 Juhasz, A.L. *et al.* (2000) Microbial degradation and detoxification of high molecular weight polycyclic aromatic hydrocarbons by *Stenotrophomonas maltophilia* strain VUN 10,003. *Lett. Appl. Microbiol.* 30, 396–401
23 Bastiaens, L. *et al.* (2000) Isolation of adherent polycyclic aromatic hydrocarbon (PAH)-degrading bacteria using PAH-sorbing carriers. *Appl. Environ. Microbiol.* 66, 1834–1843
24 Watanabe, K. (2001) Microorganisms relevant to bioremediation. *Curr. Opin. Biotechnol.* 12, 237–241
25 Kelley, I. and Cernigilia, C.E. (1995) Degradation of a mixture of high-molecular-weight polycyclic aromatic hydrocarbons by a *Mycobacterium* strain PYR-1. *J. Soil Contam.* 4, 77–91
26 Ye, D. *et al.* (1996) Degradation of polynuclear aromatic hydrocarbons by *Sphingomonas paucimobilis*. *Environ. Sci. Technol.* 30, 136–142
27 Watanabe, K. and Baker, P.W. (2000) Environmentally relevant microorganisms. *J. Biosci. Bioeng.* 89, 1–11
28 Sayler, G.S. *et al.* (1985) Application of DNA–DNA colony hybridization to the detection of catabolic genotype in environmental samples. *Appl. Environ. Microbiol.* 49, 1295–1303
29 Guo, C. *et al.* (1997) Hybridization analysis of microbial DNA from fuel oil-contaminated and noncontaminated soil. *Microbial Ecol.* 34, 178–187
30 Laurie, A.D. and Jones, G.L. (2000) Quantification of *phnAc* and *nahAc* in contaminated New Zealand soils by competitive PCR. *Appl. Environ. Microbiol.* 66, 1814–1817
31 Kanaly, R.A. *et al.* (2000) Rapid mineralization of benzo[a]pyrene by a microbial consortium growing on diesel fuel. *Appl. Environ. Microbiol.* 66, 4205–4211
32 Wildada, J. *et al.* (2002) Molecular detection and diversity of polycyclic aromatic hydrocarbon-degrading bacteria isolated from geographically diverse sites. *Appl. Microbiol. Biotechnol.* 58, 202–209
33 Adler, J. (1975) Chemotaxis in bacteria. *Annu. Rev. Biochem.* 44, 341–356
34 Harwood, C.S. *et al.* (1984) Aromatic acids are chemoattractants for *Pseudomonas putida*. *J. Bacteriol.* 160, 622–628
35 Grimm, A.C. and Harwood, C.S. (1999) NahY, a catabolic plasmid-encoded receptor required for chemotaxis of *Pseudomonas putida* to the aromatic hydrocarbon naphthalene. *J. Bacteriol.* 181, 3310–3316
36 Bhushan, B. *et al.* (2000) Chemotaxis and biodegradation of 3-methyl-4-nitrophenol by *Ralstonia* sp. SJ98. *Biochem. Biophy. Res. Commun.* 275, 129–133
37 Grimm, A.C. and Harwood, C.S. (1997) Chemotaxis of *Pseudomonas* sp. to the polyaromatic hydrocarbon, naphthalene. *Appl. Environ. Microbiol.* 63, 4111–4115
38 Ditty, J.L. *et al.* (1998) Identification of a chemotaxis gene region from *Pseudomonas putida*. *FEMS Microbiol. Lett.* 159, 267–273
39 Samanta, S.K. and Jain, R.K. (2000) Evidence for plasmid mediated chemotaxis of *Pseudomonas putida* towards naphthalene and salicylate. *Can. J. Microbiol.* 46, 1–6
40 Taylor, B.L. and Zhulin, I.B. (1998) In search of higher energy: metabolism dependent behavior in bacteria. *Mol. Microbiol.* 28, 683–690
41 Jiziore-Sasson, Y. *et al.* (1998) Metabolism is required for chemotaxis to sugars in *Rhodobacter sphaeroides*. *Microbiology* 144, 229–239
42 Timmis, K.N. *et al.* (1994) Designing microorganisms for the treatment of toxic wastes. *Annu. Rev. Microbiol.* 48, 525–557
43 Timmis, K.N. and Pieper, D.H. (1999) Bacteria designed for bioremediation. *Trends Biotechnol.* 17, 201–204
44 Sayler, G.S. and Ripp, S. (2000) Field applications of genetically engineered microorganisms for bioremediation process. *Curr. Opin. Biotechnol.* 11, 286–289
45 Ripp, S. *et al.* (2000) Bioluminescent most-probable-number monitoring of a genetically engineered bacterium during a long term contained field release. *Appl. Microbiol. Biotechnol.* 53, 736–741
46 Schell, M.A. (1983) Cloning and expression in *E. coli* of naphthalene degrading genes from plasmid NAH7. *J. Bacteriol.* 153, 822–829
47 Ogunseitan, O.A. and Olson, B.H. (1993) Effect of 2-hydroxybenzoate on the rate of naphthalene mineralization in soil. *Appl. Microbiol. Biotechnol.* 38, 799–807
48 Takizawa, N. *et al.* (1999) Nucleotide sequence and characterization of genes encoding naphthalene upper pathway of *Pseudomonas aeruginosa* Pak1 and *Pseudomonas putida* OUS82. *J. Biosci. Bioeng.* 87, 721–731
49 Khan, A.A. *et al.* (2001) Moecular cloning, nucleotide sequence, and expression of genes encoding polycyclic aromatic ring dioxygenase from *Mycobacterium* sp. strain PYR-1. *Appl. Environ. Microbiol.* 67, 3577–3585
50 Chatterjee, J. and Meighen, E.A. (1995) Biotechnological applications of bacterial bioluminescence (lux) genes. *Photochem. Photobiol.* 62, 641–650
51 Ringelberg, D.B. *et al.* (2001) Succession of phenotypic, genotypic and metabolic community characteristics during *in vitro* bioslurry treatment of polycyclic aromatic hydrocarbon-contaminated sediments. *Appl. Environ. Microbiol.* 67, 1542–1550
52 Wilson, S.C. and Jones, K.C. (1993) Bioremediation of soil contaminated with polynuclear aromatic hydrocarbons (PAHs): a review. *Environ. Pollut.* 81, 229–249
53 Guerin, T.F. (1999) Bioremediation of phenols and polycyclic aromatic hydrocarbons in creosote contaminated soil using *ex-situ* land treatment. *J. Hazard. Mater.* 65, 305–315
54 Guerin, T.F. (2000) The differential removal of aged PAHs from soil during bioremediation. *Environ. Sci. Poll. Res.* 7, 19–26
55 Bouwer, E.J. (1997) Biotreatment of PAH-contaminated soil/sediments. *Ann. New York Acad. Sci.* 829, 103–117
56 Venosa, A.D. *et al.* (1996) Bioremediation of an experimental oil spill on the shoreline of Delaware Bay. *Environ. Sci. Technol.* 30, 1764–1775
57 Lindstrom, J.E. *et al.* (1991) Microbial populations and hydrocarbon biodegradation potentials in fertilized shoreline sediments affected by the T/V *Exxon Valdez* oil spill. *Appl. Environ. Microbiol.* 57, 2514–2522
58 Sugai, S.F. (1997) Environmental influences on the microbial degradation of *Exxon Valdez* oil on the shorelines of Prince William Sound, Alaska. *Environ. Sci. Technol.* 31, 1564–1572
59 Braddock, J.F. *et al.* (1997) Enhancement and inhibition of microbial activity in hydrocarbon-contaminated Arctic soils: implications for nutrient-amended bioremediation. *Environ. Sci. Technol.* 31, 2078–2084
60 Mishra, S. *et al.* (2001) Evaluation of inoculum addition to stimulate *in situ* bioremediation of oily-sludge-contaminated soil. *Appl. Environ. Microbiol.* 67, 1675–1681
61 Margesin, R. and Schinner, F. (2001) Bioremediation (natural attenuation and biostimulation) of diesel-oil-contaminated soil in an alpine glacier skiing area. *Appl. Environ. Microbiol.* 67, 3127–3133
62 Horinouchi, M. *et al.* (2000) Removal of polycyclic aromatic hydrocarbon from oil-contaminated Kuwaiti soil. *Biotechnol. Lett.* 22, 687–691
63 Roper, J.C. and Pfaender, F.K. (2001) Pyrene and chrysene fate in surface soil and sand microcosms. *Environ. Toxicol. Chem.* 20, 223–230
64 Brunel, B. *et al.* (1988) Stability of *Bradyrhizobium japonicum*, inoculants after introduction into soil. *Appl. Environ. Microbiol.* 54, 2636–2642
65 Shaw, J.J. *et al.* (1992) Use of bioluminescence for detection of genetically engineered microorganisms released into the environment. *Appl. Environ. Microbiol.* 58, 267–273
66 Ford, C.Z. *et al.* (1999) Containment of a genetically engineered microorganism during field bioremediation application. *Appl. Microbiol. Biotechnol.* 51, 397–400