Scientific Research and Essays Vol. 5(25), pp. 4093-4106, 24 December, 2010 Special Review
Available online at http://www.academicjournals.org/SRE
ISSN 1992-2248 ©2010 Academic Journals

Review

Degradation of polycyclic aromatic hydrocarbons: Role of plasmids

Oluwafemi S. Obayori* and Lateef B. Salam

Department of Microbiology, Lagos State University, Ojo, Lagos, Nigeria.

Accepted 24 December, 2010

Polycyclic aromatic compounds are a group of highly recalcitrant organic pollutants. The initial steps in the degradation of polycyclic aromatic hydrocarbon (PAHs) involve the dihydroxylation of the aromatic ring, a step catalysed by dioxygenase enzymes. The degradation of many xenobiotic and hydrocarbon compounds is known to be mediated by plasmid encoded enzymes. In this review, an insight is given into the role of plasmid in degradation of PAHs, acquisition of degradative ability by these organisms via horizontal transfer and clustering, resulting from tranposon-mediated recombination. There is preponderance of information showing high level of plasmid involvement in the degradation of naphthalene and other 2- and 3-ring PAHs. Information on higher molecular weight PAHs is however scanty. Recent studies suggest possible involvement of plasmid in HMWPAH degradation than was previously thought. Many plasmids involved in PAH-degradation are megaplasmids, of linear configuration, encoding part or the whole genes for the complete pathways. In recent times, validation of propositions on degradative gene acquisition by horizontal gene transfer (HGT) has been obtained from field studies. HGT and transposition seems to be more chronologically linked and less fortuitously directed than previously thought. Improvement on the methods used in isolation of degraders and study of these is important, towards making a significant stride in elucidating plasmid involvement in PAH degradation.

Key words: Plasmids, degradation, polycyclic aromatic hydrocarbon.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are compounds containing carbon and hydrogen, with the carbon atoms arranged in series of adjoining six-membered benzene rings (Figure 1). Polycyclic aromatic hydrocarbons are present as natural constituents of fossil fuels. They are part of the thousands of components in petroleum products. They are also formed as a result of incomplete combustion of organic materials through pyrolysis and pyrosynthesis (Mastral and Callen, 2000) and are therefore present in relatively high concentration in products of fossil fuel combustion (Cerniglia, 1992; Kanaly and Harayama, 2000). These in combination with global transport phenomenon and increasing industrial development, result in their worldwide distribution (Jimenez and Bartha, 1996; Kanaly and Harayama, 2000; Kanaly and Harayama, 2010).

Interest in the biodegradation mechanism and environmental fate of PAHs is prompted by their ubiquitous distribution and their potentially deleterious effects on human health (Kanaly and Harayama, 2000). The chemical properties, and hence the environmental fate of PAH molecules are determined in part by both molecular size, that is, the number of aromatic rings and molecular topology or the pattern of ring linkage. PAHs are hydrophobic compounds and their persistence within the ecosystem is due largely to their hydrophobicity and low water solubility (Table 1). There is a positive correlation

*Corresponding author. E-mail: femiobayori@yahoo.com. Tel: +2348023192652.

Abbreviations: PAH, polycyclic aromatic hydrocarbon; **HGT,** horizontal gene transfer; **HMW,** higher molecular weight; **NAH,** naphthalene plasmid; **DNA,** deoxyribonucleic acid.

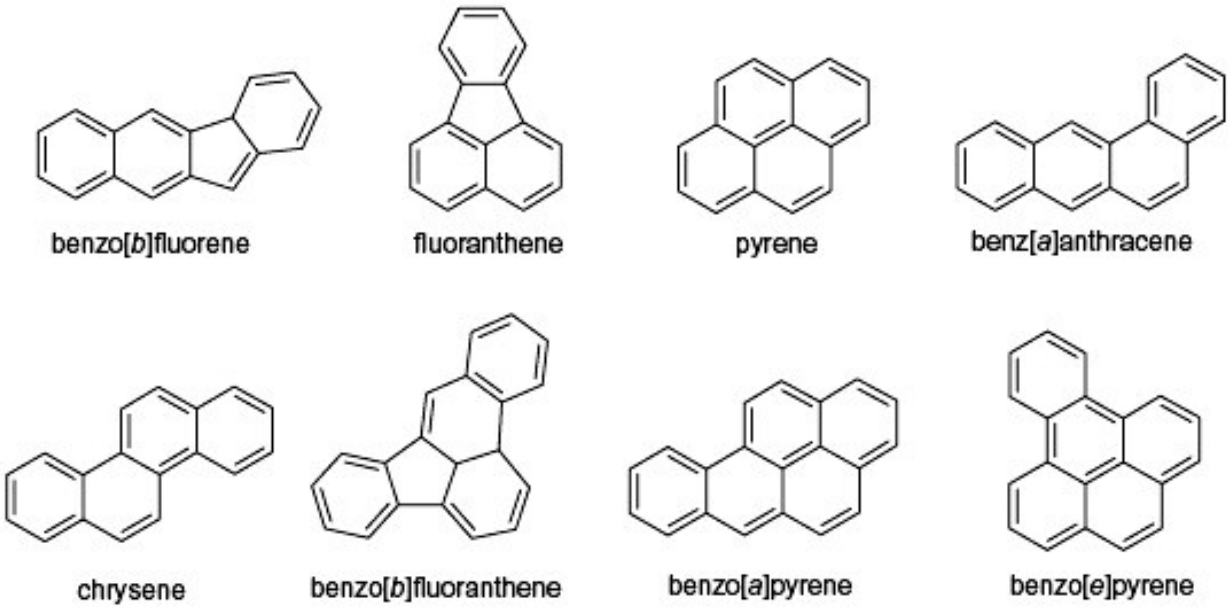

Figure 1. Structures of some selected PAHs.

Table 1. Physico-chemical properties of some PAHs.

PAH	Mass (Da)	Vapour pressure (Pa)	Log Kow	Solubility	Carcinogenicty
Naphthalene	128.18	12.0	3.58	30	-
Acenaphthene	154.20	4.02	3.92	3.6	-
Acenaphthylene	155.20	3.87	3.90	3.88	-
Fluorene	166.23	0.13	4.18	2.00	-
Phenanthrene	178.24	0.0161	4.46-4.63	1-2	-
Anthracene	178.24	0.001	4.45	0.015	-
Fluoranthene	202.26	0.001	5.22	0.25	-
Pyrene	202.26	0.0006	5.88-6.7	0.12-0.18	-
Benz[a]anthracene	228.30	$2.0x10^{-5}$	5.99	0.01	+
Chrysene	228.30	$6.08x10^{-7}$	5.01-7.10	0.0015-0.004	+
Benzo[a]pyrene	252.32	$7.0x10^{-7}$	5.78-6.5	0.001-0.006	+

Kow – octanol-water coefficient; Source: Cerniglia and Shuttleworth, 2002.

between increase in size of PAH molecules and their angularities and electrochemical stability.

PAHs are often bound to fine particles in aquatic environments, where they sediment because of their hydrophobicity. Whereas PAHs may undergo volatilization, chemical oxidation, photodecomposition and microbial degradation in water, decomposition in sediments is mainly by microbial degradation (Cerniglia, 1992). The biochemical persistence of PAHs arises from dense clouds of π-electrons on both sides of the ring structure, making them resistant to nucleophilic attack (Johnsen et al., 2005). Lower molecular weight PAHs such as naphthalene and phenanthrene are degraded rapidly in sediments, but higher molecular weight (HMW) PAHs such as pyrene, chrysene, benzo(a)anthracene and benzo(a)pyrene are more recalcitrant (Mrozik et al., 2003).

A wide array of microorganisms including fungi, algae and bacteria are known to degrade PAHs. However, bacteria play by far the most important role in complete mineralization. Fungi on the other hand mainly biotransform PAHs, namely, detoxification to less or non-toxic metabolites which can then be acted upon by other organisms (Cerniglia, 1992). Most of the bacteria known to degrade high molecular weight PAHs are actinomycetes, belonging to the genera *Mycobacterium*, *Rhodococcus* and *Gordonia* (Krivobok et al., 2003). The initial step in the aerobic catabolism of an aromatic molecule by bacteria, occurs via oxidation of the PAH to a dihydrodiol by a multicomponent enzyme system. This

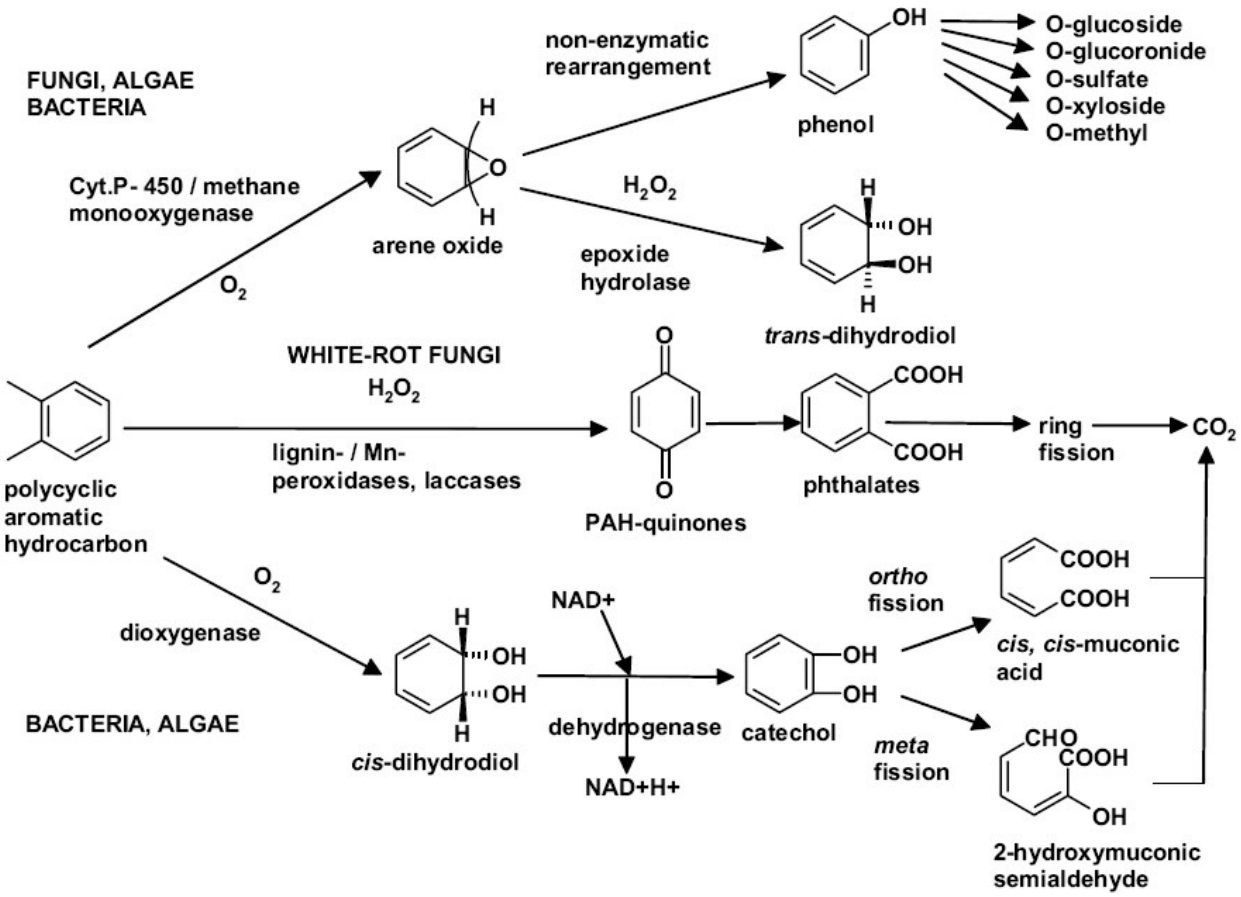

Figure 2. Pathways of microbial degradation of PAHs.

hydroxylated intermediate may then be processed to a central intermediate catechol or protocatechuate, which is cleaved through the ortho or meta cleavage type of pathways (Figure 2). In polynuclear aromatic compounds like PAHs, there is sequential cleavage of the rings via dihydroxylation and cleavage leading to more intermediates and ultimate conversion to intermediates of the tricarboxylic acid cycle (van Der Meer et al., 1992; Kanaly and Harayama, 2000).

DEGRADATIVE PLASMIDS OF PAH DEGRADERS

A bacterium needs the appropriate catabolic genes in order to be a degrader of a compound. Many of the genes involved in the degradation of PAHs are often located on plasmids (Johnsen et al., 2005). These extra-chromosomal elements first came to reckoning with the discovery of their involvement in the spread of bacteria resistance to antibiotics (Foster, 1983). Plasmids that carry structural genes that code for the degradation of many naturally occurring organic compounds and xenobiotics, are referred to as degradative or catabolic plasmids. The first plasmids that were discovered were predominantly circular plasmids (Hardy, 1981; Day, 1982) but reports abound today of plasmids with linear configuration and a number of these have been reported in degraders of xenobiotics and other recalcitrant pollutants (Shimizu et al., 2001; Stecker et al., 2003; Broker et al., 2004).

The role of plasmids in the evolution of bacteria populations in the environment cannot be overemphasized. The presence of PAH-degradative genes on mobile genetic elements has been fingered as an indication of easy spreading of PAH-catabolic abilities among bacteria in polluted soil, as a result of conjugative transfer (Johnsen et al., 2005). This is further buttressed by the fact of sequence identity of genes among strains (Wackett and Hershberger, 2001). A plasmid may encode a complete degradative pathway or partial degradative step. Some other plasmids code for enzymes that have specificity for several substrates. For example, the genes encoding the upper and lower pathways of naphthalene in the NAH plasmids of several pseudomonads have broad specificities, allowing the host to grow on several two and three -ring PAHs, as sole carbon and energy sources (Foght and Westlake, 1996). Some of the best reported plasmid-bearing PAH degraders are listed in Table 2.

Table 2. Selected PAH degrading bacterial plasmids and their hosts.

Strain	Plasmid	Substrate	References
Pseudomonas putida strains	NAH7	Naphthalene	Dunn, 1973
Pseudomonas putida NCIB9816	pDTG1	Naphthalene	Kurkela et al., 1988
Pseudomonas strain C18	pUC18	Dibenzothiophene naphthalene, Phenanthrene	Denome et al., 1993
Pseudomonas sp. strain 112		Naphthalene	Fuenmayor et al., 1998
Burkholderia strain R007		Naphthalene, phenanthrene	Laurie and Lhoyd-Jones, 1999
Sphingomonas aromaticivorans F199	pNL	Phenanthrene	Romine et al., 1999
Sphingomonas strain KS14	pKS14	Phenanthrene	Cho and Kim, 2001
Staphylococcus sp. PN/Y	pPNY	Phenanthrene	Mallick et al., 2007
Terrabacter sp. DBF63	pDBF1	Fluorene	Habe et al., 2005
Sphingomonas sp. HS362	p4	Phenanthrene	Hwa et al., 2005
Mycobacterium sp. KMS		Pyrene	Miller et al., 2007
Mycobacterium sp MC		Pyrene	Miller et al., 2007
Mycobacterium sp. PYR-GCK		Pyrene	Miller et al., 2007
Pseudomonas sp. ARP26		Phenanthrene	Coral and Karagol, 2005
Pseudomonas sp. ARP28		Phenanthrene	Coral and Karagol, 2005
Pseudomonas sp.		Anthracene	Kumar et al., 2010
Beijerinkia sp.	pKG2	Phenanthrene	Kiyohara et al., 1983
Pseudomonas fluorescens strain LP6a	pLP6a	Naphthalene anthracene, phenanthrene	Foght and Westlake, 1996

Table 3. Genes borne on the NAH7 plasmid and enzymes encoded by them.

Substrate	Gene	Encoded protein or function
Naphthalene (upper pathway)	nahAa	Reductase
	nahAb	Ferredoxin
	nahAc	Iron sulfur protein large subunit
	nahAd	Iron sulfur protein small submit
	nahB	cis-Napthalene dihydrodiol dehydrogenase
	nahF	salicyaldehyde dehydrogenase
	nahC	1,2-Dihydroxynaphthalene oxygenase
	nahE	2-Hydroxybenzalpyruvate aldolase
	nahD	2-Hydroxychromene-2-carboxylate isomerase
Salicylate (lower pathway)	nahG	Salicylate hydroxylase
	nahT	Chloroplast-type ferredoxin
	nahH	Catechol oxygenase
	nahI	2-Hydroxymuconic semialdehyde dehydrogenase
	nahN	2-Hydroxymuconic semialdehyde dehydrogenase
	nahL	2-Oxo-4-pentenoate hydratase
	nahO	4-Hydroxy-2-oxovalerate aldolase
	nahM	Acetaldehyde dehydrogenase
	nahK	4-Oxalocrotonate decarboxylase
	nahJ	2-Hydroxymuconate tautomerase
Regulator for both operons	nahR	Induced by salicyylate

DEGRADATIVE PLASMIDS ENCODING NAPHTHALENE DEGRADATION

Naphthalene is the simplest, the most easily degraded and the best-studied among the PAHs. The association of plasmids with the degradation of naphthalene by bacteria has been well reported in the literature and was the subject of a remarkable review by Yen and Serdar

(1988). All the genes involved in the degradation of naphthalene by *Pseudomonas putida* PpG7 are now known to be plasmid borne (Table 3). Dunn and Gunsalus (1973) reported for the first time, the involvement of plasmids in the degradation of PAHs. The genes for the initial step in the oxidation of the naphthalene were localized on the NAH plasmid in *Pseudomonas* PpG7. This gene was found to be transmissible. The authors suggested that plasmids might play an important role in the evolution of metabolic diversity among pseudomonads. Dunn et al. (1980) isolated and characterized two catabolic plasmids coding for the degradation of naphthalene in *P. putida* strains. The plasmids were transmissible, belonged to the p7 incompatibility group and coded for naphthalene degradation via salicyclate and catechol, then, by the catechol meta-cleavage pathway. Enzymes for the meta-cleavage of catechol were found to be constitutive, in mutant carrying the plasmid NAH, pND140 and pND160. The enzymes involved in the conversion of naphthalene to catechol were found to be inducible during growth on salicyclate. Thus, the authors concluded that these enzymes belonged to separate operon or operons (Austen and Dunn, 1980).

Yen and Gunsalus (1982) demonstrated that the genes encoding the enzymes of the first 11 steps of the naphthalene oxidation pathway are located on the NAH7 plasmid. In plasmid NAH7, the naphthalene catabolic genes are organized into two operons; nah and sal. The naphthalene oxidation genes are organized in two operons. The first operon includes genes nahABCDEF, coding for the conversion of naphthalene to salicylate, and the second operon includes genes nahGHIJK, coding for the oxidation of salicylate via the catechol meta-cleavage pathway. Further progress was made in unraveling the nature and functions of the genes encoded on this degradative plasmid when Yen and Gunsalus (1985) characterized a series of clustered NAH7 plasmid mutations, which defined the regulatory gene nahR. The authors showed conclusively that the two catabolic operons in NAH7 are controlled by a positive regulator gene, nahR, that is located immediately upstream of the nahG gene. Induction of the operons is controlled not by naphthalene but by its metabolite, salicylate. Schell and Wender (1986) identified the nahR gene product and nucleotide sequence required for the activation of the sal operon of NAH7. You et al. (1991) sequenced the intergenic region spanning genes nahG and nahH. The NahR-nahG regulatory gene function was later demonstrated to be highly conserved (Park et al., 2002). Apart from the NAH plasmids, several other plasmids were equally reported early enough. Zuniga et al. (1981) demonstrated that the ability of *P. putida* PMD-1 to utilize naphthalene and salicylic acid as sole carbon sources was due to the presence of plasmid pMWD-1 of 110 megadaltons and that the pathway was meta-cleavage. It was shown that, the plasmid was required for the degradation of salicyclate while conversion of naphthalene to salicylate was chromosomally borne.

Using a rapid method, Connors and Barnsley (1982) showed that, three naphthalene degraders of *Pseudomonas* strain, NCIB 9816, *Pseudomonas* strain PG and ATCC 17483 each harbor more than one plasmid. The largest of the plasmids NAH2 and NAH3 in NCIB 9816 and PG were found to hybridize with NAH, although quite different. Later, plasmid deoxyribonucleic acid (DNA) from *P. putida* NC1B 9816 was cloned in *Escherichia coli* and the transformant was used to transform naphthalene to salicycaldehyde, salicyclic acid and an unknown product (Cane and William, 1982). Schell (1983) demonstrated that the genes for the enzymes responsible for conversion of naphthalene to 2-hydroxymuconic acid (nahA through nahI) are contained on a 25-kb EcoRI fragment of an 85 kb NAH plasmid of *P. putida*. A set of naphthalene degradation genes on the *Pseudomonas* plasmid NAH7 was shown to be part of a defective transposon which becomes mobile in the presence of a Tn4653 transposase (Tsuda and Iino, 1990). Foght and Westlake (1996) localized the gene for PAH degradation by *Pseudomonas fluorescence* LP6a which utilized naphthalene, phenanthrene anthracene and methylnaphthalene as sole carbon and energy sources on a 63 kb plasmid (pLP6a). The plasmid was hybridized to the NAH7 and pWW60 but had different restriction endonuclease patterns. This plasmid exhibited reproducible spontaneous deletion of a 38 kb region containing the degradative genes.

THE NAH GENES ARE HIGHLY VERSATILE

The naphthalene dioxygenase enzyme encoded by the NAH7 genes is known today to be a highly versatile enzyme system, encoding a wide range of reactions (Ensley et al., 1983; Parales et al., 2002). The NAH7 plasmid belongs to the IncP-9 incompatibility group. It is one of the three completely sequenced IncP-9 plasmids alongside pWWO (Williams and Murray, 1974) and pDTG1 (Kurkela et al., 1988). The plasmids in this group are mainly large self-transmissible plasmids, associated with degradation, and antibiotic and toxic metal resistance markers. The first evidence of the versatility of the NAH plasmid encoded genes was provided independently by two research groups (Menn et al., 1993; Sanseverino et al., 1993). This led Sanseverino et al. (1993) to conclude that maintaining and monitoring one catabolic bacterial population may be sufficient for degradation of a significant fraction of PAHs in contaminated soil. Kiyohara and Nagao (1978) and Barnsley (1983) had proposed that enzymes other than NahA catalysed dioxygenation in phenanthrene metabolism but findings by Sanseverino et al. (1993) confirmed that the enzymes were indeed NahA and were borne on NAH7- like plasmid.

Menn et al. (1993) produced the first report which provides direct biochemical evidence that the

naphthalene plasmid degradative enzyme system is involved in the degradation of higher-molecular-weight polycyclic aromatic hydrocarbons other than naphthalene. *Pseudomonas fluorescens* SR, which contained an NAH7-like plasmid (pKA1), and *P. fluorescens* 5R mutant 5RL containing a bioluminescent reporter plasmid (pUTK21) which was constructed by transposon mutagenesis were used. Polymerase chain reaction (PCR) mapping confirmed the localization of lux transposon Tn4431 300 bp downstream from the start of the nahG gene. 2-hydroxy-3-naphthoic acid and 1-hydroxy-2-naphthoic acid, were recovered and identified from *P. fluorescens* 5RL as biochemical metabolites from the biotransformation of anthracene and phenanthrene, respectively.

Plasmids that encode the degradation of naphthalene have also been found to be involved in the pathways of dibenzothiophene transformation, and it has been suggested that all the dibenzothiophene plasmids were actually naphthalene plasmids which are closely related to NAH7 (Eaton, 1994). Denome et al. (1993) completely sequenced the Dox genes in *Pseudomonas* strain C18 and showed that the same genes are responsible for the upper pathway of naphthalene degradation. The genes were found to be on a 75-kb plasmid doxABDFGHIJ. The authors concluded, therefore, that a single genetic pathway controls the metabolism of dibenzothiophene, naphthalene and phenanthrene in strain C18. At about the same time, Simon et al. (1993) reported that the DNA sequence for genes of nah (nahAb, nahAc and nahAd) were namely identical with the doxABD genes. Laurie and Lloyd-Jones (1999) reported the description of a divergent set of PAH catabolic genes, the phn genes in *Burkholderia* sp. strain RP007 which are isofunctional to the classical NAH-like genes but show low homology. The genes encoded the entire upper pathway of naphthalene and phenanthrene degradation. The gene nahY which code for the membrane protein that is a chemoreceptor for naphthalene or naphthalene metabolite is encoded by the NAH7 plasmid of *P. putida* G7 (Grimm and Harwood, 1997, 1999). Samanta and Jain, (2000) reported that *P. putida* RKJI possesses an 83-Kb plasmid for naphthalene metabolism through salicyclate. Nap – Sap+ mutant was chemotactic towards only salicylate.

Romine et al. (1999) determined the complete 184, 457 bp sequence of aromatic catabolic plasmid pNL1 from *Sphingomonas aromaticivorans* F199. This represents the first report of complete sequence of a conjugative plasmid that encodes pathways for the complete catabolism of aromatic organic compounds. Nearly half of the pNL DNA encoded genes are suspected to be involved in either catabolism or transport of aromatic compounds. The remainder of the plasmid appears to encode functions associated with plasmid replication, maintenance or transfer. The genes for naphthalene degradation in *S. aromaticivorans* F199, a broad-spectrum xenobiotic degrader, are encoded on a large plasmid (pNL1) which is circular (Basta et al., 2004). Dennis and Zylstra (2004) completely sequenced and constructed the physical map pDTG1 plasmid of *P. putida* NCIB 9816. Notwithstanding the seeming ubiquity of plasmid-borne naphthalene degradation genes, there are strains in which all the genes necessary for the catabolism of naphthalene are chromosomally borne, such as naphthalene degradation in *Rhodococcus* sp. strain B2 proceeds through salicyclate and gentisate but not by plasmid (Grund et al., 1992). Also, in *P. putida* OUS82 (Kiyohara et al., 1994; Takizawa et al., 1994) and *Pseudomonas stutzeri* AN10 (Bosch et al., 1999; 2000), the genes are entirely chromosomally borne. The presence of the genes for both the upper pathway and lower pathway of naphthalene degradation in some bacteria further gives credence to the notion that lateral gene transfer and genetic recombination may have played an important role in the development of such versatile metabolic pathways.

It is also interesting that among strains that carry plasmids, wide divergence from classic types are sometimes observed. For instance, Rossello-Mora et al. (1994) studied 11 naphthalene-degrading *P. stutzeri*, and found that only one carried a plasmid encoded pathway. The genes in this strain were also different from those of NAH7. *Pseudomonas* strain U2 carried a plasmid for the gentisate pathway utilization of naphthalene, instead of the meta-cleavage pathway of catechol. The gene order negAa-nagh-nagH-ngAb-nagA-nagAd-nagB-nagF was typical for strains that used this pathway (Fuenmayor et al., 1998), quite different from that of NAH7 and its closely related naphthalene degraders.

Bosch et al. (1999) showed that the entire catabolic module of *P. stutzeri* AN10 was recruited from other microorganisms and a short period of time has elapsed after its incorporation within the *P. stutzeri* AN10 genome. Further investigations gave credence to the notion that, catabolic modules are recruited by transposition events and recombination among tnPA-like genes and subsequent rearrangement and deletions on non-essential DNA fragments allowed the formation of actual catabolic pathways (Bosch et al., 2000).

PLASMIDS INVOLVED IN THE DEGRADATION OF PHENANTHRENE AND OTHER THREE-RING PAHS

Reports abound on the involvement of plasmids in phenanthrene degradation. As far back as 1983, a *Beijerinkia* sp. growing on phenanthrene, biphenyl and other PAHs was found to possess two plasmids: PKG1 and PKG2 (147 and 20.8 KDa, respectively) and the degradation of phenanthrene and biphenyl was localized on PKG2 (Kiyohara et al., 1983). Also, it was demonstrated that a phenanthrene utilizing Flavobacterium isolated from Chesapeake Bay sediment harbored a 34 MDa plasmid (Okpokwasili et al., 1984). Degradation of phenanthrene by *Mycobacterium* strain BG1 was found to

be via meta-cleavage of protocatechuate and plasmid associated (Guerin and Jones, 1988). The three plasmids involved were found to be of sizes 21, 58 and 77 MDa. Degradation of phenanthrene by *Alcaligenes faecalis* AFK2 is encoded on plasmid PHK2 (42.5 kb) (Kiyohara et al., 1990).

Cho and Kim (2001) detected a 500 kb plasmid in *Sphingomonas* strain K514 capable of mineralizing phenanthrene to carbon dioxide. This plasmid was much larger than the NAH plasmid, that played a major role in naphthalene degradation. Phenanthrene degradation in two *Pseudomonas* strains ARP26 and ARP28 from polluted soils in Turkey have been reported to be plasmid mediated, although the plasmids involved are small plasmids (Coral and Karagoz, 2005). Phenanthrene degrading bacterium *Sphingomonas* sp. HS362 was found to carry five plasmids, and degradative ability was localized on plasmid P4 (Hwa et al., 2005).

Okoro et al. (2009) isolated five bacteria that were able to grow on phenanthrene and dibenzothiophene from produced water. The plasmids (<23.1 kb) were however determined by curing, not to be involved in the degradation of these compounds by the isolates, namely *Acinetobacter lwoffi*, *Enterobacter* sp., *Pseudomonas* sp. Corynebacterium, *Vibro* sp. and *Pseudomonas aeruginosa*. Mallick et al. (2007) reported that, the genes encoding the degradation of phenanthrene in *Staphylococcus* sp. strain PN/Y are plasmid-borne. The plasmid (pPHN) is a large plasmid (112 kb) and encodes the enzyme for the meta-cleavage of 2 hydroxy-1-naphthoic acid with the formation of a unique intermediate trans -2, 3-dioxo-5-(2- hydroxyphenyl) pent-4-enoic acid. As there had been no previous report of gene for dioxygenase activity in the data base of the genes of *Staphylococcus*, the authors suggested that horizontal transfer may have been instrumental to the acquisition of PAH-metabolizing ability by strain PN/Y.

Notwithstanding the plethora of reports on degradation of phenanthrene by plasmid mediated pathways; however, a number of reports have also indicated chromosomally mediated phenanthrene degradation by bacteria. For instance, it was shown that the genes for phenanthrene degradation in *Commamonas testosteroni* strain are chromosomally borne (Goyal and Zylatra, 1996, 1997). It is also noteworthy that the genes for the catabolism of phenanthrene by *Nocardiodes* sp. strain KP7 were found to be chromosomally borne (Saito et al., 2000).

A new remarkable vista in the role of plasmid in the degradation of PAHs was opened when Habe et al. (2005) localized the genes for catabolism of fluorene in *Terrabacter* sp. DBF63 on a linear plasmid (pDBF1). A 70.631 Kb region of the plasmid was found to include B-keto adipate pathway genes. The significance of this finding is in the fact that, these genes are usually located on chromosomes in proteobacteria and actinobacteria (Harwood and Parales, 1996; Iwagami et al., 2000; Konig et al., 2004). Furthermore, the authors for the first time elucidated all catabolic genes for conversion of fluorene to TCA cycle intermediates and also found separate pcaD and pcaC genes but not the merged pcaL gene within the pca gene cluster of an actinobacteria species. The location of the pca gene cluster on the linear plasmid and the insertion sequences around the pca gene cluster were interpreted by the authors to suggest that B-keto adipate pathway genes may be spread widely among bacterial species via horizontal transfer or transpositional events. The pathway proposed for fluorene degradation by *Terrabacter* sp. DBF63 and encoded on a linear plasmid (pDBF1) is shown in Figure 3.

Only very few acenaphthene degraders have been reported in the literature (Komatsu et al., 1993; Selifonov et al., 1993; Shi et al., 2001; Kouzuma et al., 2006), and there are no reports yet of plasmid involvement in the degradation of PAH by these organisms. The involvement of plasmids in the bacterial degradation of anthracene has been reported in the literature (Ilori, 1998; Kumar et al., 2010), but information on the organization of genes on these plasmids are sparse, except in the cases of those associated with the Nah genes (Sanseverino et al., 1993; Menn et al., 1993).

PLASMIDS INVOLVED IN THE DEGRADATION OF PYRENE AND OTHER HMW PAHS

Although plasmids have been well reported in naphthalene and some of the other low molecular weight PAHs such as fluorene, phenanthrene and anthracene, similar reports are sparse and far between for the high molecular weight PAHs such as pyrene, fluoranthene, benzo(a)pyrene, benzo(a)anthracene and others. Between 1988 when the first pyrene degrader was reported and now, most studies have found genes for pyrene degradation to be localized on chromosomes (Heitkamp et al., 1988; Khan et al., 2001; Krivobok et al., 2003; Kim et al., 2007). But in recent years, reports have focused on this area and there have been some interesting findings. Sho et al. (2004) isolated a *Mycobacterium* sp. strain S65 from a set of fuel contaminated sit. This isolate, which was able to grow on pyrene, phenanthrene and fluoranthene, as sole carbon and energy sources, was found to have a large plasmid. But further study by hybridization to the nidA gene indicated that, the plasmid was not associated with pyrene degradation.

A major breakthrough in the study of plasmids in pyrene degraders was made when Miller et al. (2007) demonstrated plasmids in *Mycobacterium* sp. KMS (150 - 225 kb) *Mycobacterium* species MC (150-215 kb) and *Mycobacterium* sp. PYR-GCK (300 - 350 kb) *Mycobacterium* sp. JLS and the well studied *Mycobacterium vanbaaleni* PYR-1 possessed no plasmid. It is noteworthy that these are isolates which

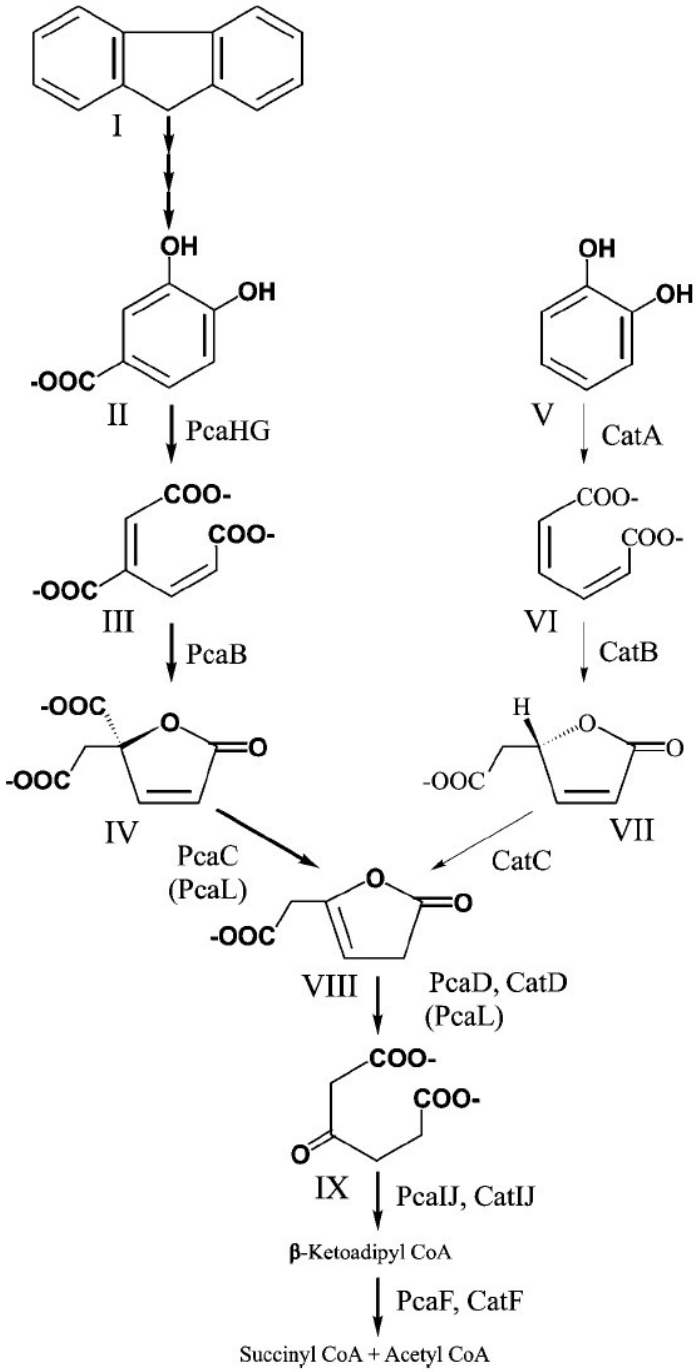

Figure 3. The pathway of fluorene degradation by Terrabacter sp. strain DBF63 (represented with bold arrows). Compound designations: I, fluorene; II, protocatechuate; III, b-carboxy-cis,cis-muconate; IV, ccarboxymuconolactone; V, catechol; VI, cis,cis-muconate; VII, muconolactone; VIII, b ketoadipate enol-lactone; IX, b-ketoadipate. Enzyme designations: PcaHG, protocatechuate 3,4-dioxygenase; PcaB, b-carboxy-cis, cis-muconate cycloisomerase; PcaC, ccarboxymuconolactone decarboxylase; PcaD and CatD, b-ketoadipate enol-lactone hydrolase; PcaL, a fused c-carboxymuconolactone decarboxylase/b-ketoadipate enol-lactone hydrolase; CatA, catechol 1,2-dioxygenase; CatB, muconate cycloisomerase; CatC, muconolactone isomerase; PcaIJ and CatIJ, b-ketoadipate succinyl CoA transferase; PcaF and CatF, b-ketoadipyl CoA thiolase (Habe et al. 2005).

had been previously taken for granted as non-plasmid bearers. It would thus appear that, the choice of method is very important in the detection of plasmids, as most large plasmids are likely to escape detection using routine techniques used for small plasmids. It is not unlikely that plasmids are more involved in the degradation of HMW PAH than previously thought. Lin and Cai (2008) reported the isolation of plasmid from a pyrene degrading consortium made up of two strains; *Bacillus cereus* Py5 and *Bacillus megaterium* Py6. Pyrene degradation was localized on the plasmid, after plasmid transformed *E. coli* was shown to degrade pyrene. However, there are no reports yet on the involvement of plasmids in the degradation of pyrene by Pseudomonads. Obayori et al. (2008) could not also detect plasmids in the three pyrene degrading *Pseudomonas* strains isolated from polluted tropical sites in Lagos, Nigeria in spite of the remarkable versatility of these isolates.

Benzo(a) pyrene is a highly carcinogenic PAH and is classified by the United States Environmental Protection Agency (EPA) as a priority pollutant. Benzo(a)pyrene biotransformation, cometabolism, mineralization in soil and degradation by organisms isolated on other PAHs, have been well reported (Kanaly and Harayama, 2000; Moody et al., 2004; Rentz et al., 2005; Rentz et al., 2008; Schneider et al., 1996) but isolation on this substrate has only been recently reported. According to Kanaly and Harayama (2010), benzo(a)pyrene is not known to be utilized as a sole source of carbon and energy by any bacteria and significant biodegradation appears to require bacterial cooperation, although the mechanisms are still unclear. But a recent report by Lily et al. (2010) showed that a new isolate, *Bacillus subtilis* BMT4i could degrade Benzo(a)pyrene and provided incontrovertible evidence, that the genes involved are borne on the chromosome rather than plasmid. This is the only report on research directed at attempting to link Bap degradation with plasmids and it does not appear much could be achieved in this area, until much is known about organisms that can utilize Bap as a sole source of carbon and energy.

ROLE OF PLASMIDS IN THE EVOLUTION OF DEGRADATIVE ABILITY

Plasmids play a very pivotal role in the evolution of degradative abilities among microorganisms. They allows bacterial populations to access the horizontal gene pool for adaptive traits that might be useful for their survival under local selective pressure, imposed by organic chemical challenges. Plasmids also promote genetic variation, act as vehicle for genetic recombination, and owing to mechanisms such as post-segregational killing (psk) of plasmid-free cells, stable partitioning (par) of plasmids into daughter cells and multimer-resolution systems (MRS) promote stable transfer/inheritance of the new phenotypic traits (Easter et al., 1998; Gerdes et al., 2004; Sorensen et al., 2005; Van Melderen, 2002). Among the mechanisms of genetic adaptation by micro-organisms to organic pollutants, genetic transfer plays a prominent role. The importance of horizontal gene transfer (HGT) in bacterial evolution is corroborated by the realization that, as much as 24% of the *E. coli* genome was acquired through HGT (Lawrence and Ochman, 2002). More specifically, catabolic plasmids play a significant role in the horizontal spread of existing catabolic pathways, as well as in the construction of new ones (Top and Springael, 2003). These plasmids are self-transmissible plasmids (Mob+, Tra+) with broad host range specificities. Furthermore, they are usually flanked at non-essential sites by insertion sequence (IS) elements, which not only aid in the recruitment of catabolic genes by the plasmid but also increase the possibility of further exchange of the genes between different hosts and replicons.

Top and Springael (2003) highlighted four key findings emanating from previous studies on the role of HGT in evolution of degradative abilities among bacteria. Firstly, bacteria originating from diverse locations encode evolutionarily related catabolic genes and gene clusters. Secondly, the phylogeny of the catabolic genes is at variance with that of the 16S rRNA genes of the corresponding hosts. Thirdly, catabolic genes encoding degradation of organic pollutants are often borne on plasmids and transposons. Lastly, evolutionarily related catabolic genes and entire gene modules are involved in the degradation of structurally similar but different xenobiotic compounds. The observation that slightly differs from homologous operons, encoding for catabolic pathways have been found frequently in phylogenetically distant organisms, and also suggests the occurrence of extensive HGT (Bosma et al., 2001). For example, exact copies of the xyl operons, encoding the enzyme for toluene/xylene degradation, have been detected on plasmids of different incompatibility groups (Sentchillo et al., 2000). In addition, the average G+C contents of both the upper naphthalene degradation pathway, from pDTG1, and the upper xylene degradation pathway, from pWW0, was found to be significantly different from their respective plasmid G+C contents (53/56%, 49/59%), indicating that these regions were imported from a different organism (Dennis and Zylstra, 2004; Harayama, 1994; Greated et al., 2002).

The emergence of class I and class II transposons, which often border catabolic plasmids encoding some phenotypic determinants, has further widened the catabolic versatility and adaptability of catabolic plasmids. Class I transposons are composite transposons in which the catabolic genes are flanked by two copies of very similar IS elements in direct or inverted orientation. Class II transposons are non-composite transposons that carry short terminal inverted repeats and transpose by the replicative mode, involving transposases and resolvase (Top et al., 2002). Insertion sequences have been implicated in DNA rearrangement, gene transfer, and in activation/

inactivation of silent genes (Van der Meer et al., 1992). The recruitment of catabolic genes from different organisms by the transposons, the modular assembly of the recruited catabolic gene clusters by transposons into a promiscuous self-transmissible, broad host range plasmid, and their mediation of further exchange of the genes between different hosts and replicons are strong indications of the significance of transposons in the evolution of degradative abilities among bacteria. An overview of various findings from laboratory and field studies corroborating and illustrating the roles of catabolic plasmids in the evolution of degradative abilities among PAHs-degrading bacteria is summarized herein. The role of plasmids in the adaptation of bacteria to life in polluted, particularly hydrocarbon impacted matrices, cannot be overemphasized. This is obviously because, they are highly mobile genetic elements which can easily be transferred horizontally within and between populations in the environment (Atlas, 1981; Leahy and Colwell, 1990). Early in the study of catabolic plasmids, it was suggested that genes for catabolic steps in degradative pathways, might evolve piecemeal in different organisms, followed by their assemblage in the form of non-transmissible plasmids by transposition and recombination (Wheelis, 1975; Chakrabarty, 1978). Ferrell (1979) also suggested that transfer of structural genes occur without concomitant transfer of their regulatory genes, so that the genes encoding enzymes for the pathway are regulated as a single unit.

Several reports have shown that, the meta-cleavage pathway is highly conserved among aromatic hydrocarbon degraders (Cane and Williams, 1982, 1986; Ghosal and Gunsalus, 1987; Assinder and Williams, 1988; Harayama et al., 1993). Platt et al. (1995) reported the complete sequence of the nahOM gene for the acetaldehyde dehydrogenase (acylating) and the 4-hydroxy-l-oxovalerate aldolase from the meta-pathway operon of the naphthalene catabolic plasmid pWW60-22 from *Pseudomonas* sp. NClMB9816. The authors provided incontrovertible evidence that, the nahNLOMK genes of pWW60-22 are both homologous to and in the same order as the equivalent dmpDEFGH and xylFJQKI of the phenol/methylphenol, and toluene plasmids pVII50 and pWWO, respectively. The finding showed that the genes of the meta pathway share a common ancestry and that recombination could play a very important role in the evolution of lower pathway operons in the meta pathway genes.

Evidences abound that many of the plasmids that are involved in the degradation of PAHs have common ancestry. For instance, it was shown by Stuart-Keil et al. (1998) that the pCg1, a naphthalene catabolic plasmid carried by *P. putida* Cg1, is homologous to the archetypal naphthalene catabolic plasmid, pDTG1, in *P. putida* NCIB 9816-4. The plasmid was later demonstrated to possess a special conjugal transfer region with genes enhancing transfer (Park et al., 2003). Chrysene degrading organism *Sphingomonas* sp. Strain CHY-1 possessed two distinct loci containing clustered catabolic genes with strong similarities to corresponding genes found in *Novosphingobium aromaticivorans* F199 (Demaneche et al., 2004).

The catabolic genes of F199 had an unusual arrangement, in that the genes predicted to participate in the degradation of monoaromatic hydrocarbon were interspersed with genes potentially involved in biphenyl or PAH catabolism (Romine et al., 1999). Furthermore, multiple copies of the genes that potentially encoded ring-hydroxylating dioxygenase terminal components were identified. Genes that encode enzymes associated with the degradation of biphenyl, naphthalene, m-xylene and p-cresol were predicted to be distributed among 15 gene clusters. But more importantly, several genes associated with integration and recombination were identified in the replication region, suggesting therefore that pNL1 is able to undergo integration and excision events with the chromosome and/or other portions of the plasmid. It was later shown that a phenanthrene degrading *Sphingobium* strain carry catabolic genes similar to F199 and three distinct oxygenases has salicyclate hydroxylase activity (Pinyakong et al., 2003b, 2004). Proteins presumably involved in transport of aromatic compounds across the cell via efflux pumps were also identified. It is noteworthy that Neher and Lueking (2009) that similarly localized the gene involved in the diffusion of naphthalene into the cell in *P. fluorescence* on plasmid. The fact that plasmid such as the three IncP-9 plasmids, pWW0, pDTG1 and NAH7, exhibit extensive homology in replication, partitioning and transfer loci seems to point to the common origin of these plasmids. Sevastsyanovich et al. (2008) suggested that some IncP-7 plasmids are the products of recombination between plasmids of different IncP-9 sub-groups, and that recombination between related plasmids might be more common than previously assumed for incompatible replicons. The implication of this for the evolution of degradative pathways by these organisms in trying to cope with new substrates which are slightly more complex than the ones to which they are already adapted cannot be overemphasized. Herrick et al. (1997) demonstrated natural horizontal transfer of naphthalene dioxygenase genes among indigenous bacteria in a coal tar contaminated site. They thus suggested that horizontal transfer of biodegradation genes may play a role in the adaptation of bacterial populations to organic contaminant compounds. Furthermore, they pointed out that plasmid modification after transfer is effected by transposon.

More recent findings relying on genomic resources from diverse sources have strengthened the conviction on this score. Ma et al. (2006) showed that large plasmids played very important role in horizontal gene transfer among 22 PAH degraders isolated from Antarctica soils. All these isolates were *Pseudomonas* except a strain of *Rahnella*. The plasmids were found to

be large (60 to 80 kb) and self-transmissible. Some of the strains contained some small plasmids in addition to the large ones. The genes for dioxygenation of naphthalene or phenanthrene in all the isolates were localized on the plasmid by plasmid curing and southern blotting and hybridization. The possibility of horizontal gene transfer among the strains were predicated on lack of phylogenetic correlation between dioxygenase and its host; the fact that different species contained very similar and in some cases, the same naphthalene dioxygenase (NDO) genes. Likewise, some strains with very similar NDO genes exhibited highly divergent plasmid structures. Two separate pyrene degradation gene clusters were found in strain S65. One of these two loci, the pdo locus also encoded an 1S3 like transposase upstream of pdoB (Sho et al., 2004). The homology of these loci with the naphthalene inducible pyrene dioxygenase gene nidA from *M. vanbaaleni* strain PYR-1 and the presence of transposase, lend further credence to the notion of involvement of transposons in evolution of PAH catabolic genes. Wilson et al., (2003) showed that a group of naphthalene degraders from a PAH contaminated hillside were phenotypically and genotypically distinct from naphthalene degrading organisms isolated from adjacent, more highly contaminated seep sediments.

Recently, Christie-Oleza et al. (2008) described an insertion sequence ISPst9 in *P. stutzeri* AN10, this insertion sequence was found to transpose in multiple copies and capable under pressure of causing mutation of nahH (catechol 2, 3- dioxygenase encoding gene). Further studies (Christie-Oleza et al., 2009) showed that, conjugative interaction plays a crucial role in activating ISPst9 transposition. It would thus appear that, genetic exchange by conjugation in this organisms triggers transposition, thus increasing the possibility of transfer of such acquired genetic element to the chromosome. This gives weight to the argument that evolution of pathway is under the influence of the two closely linked mechanisms of horizontal transfer and recombination by transposition.

CONCLUSIONS

From the foregoing, certain important conclusions can be drawn. Firstly, plasmid involvement in the degradation of PAHs is widespread, with more report on lower molecular weight PAHs, particularly naphthalene. Secondly, many of the plasmids are megaplasmids with linear configuration and demonstrate great diversity in arrangement and clustering. Thirdly, horizontal transfer and subsequent transposon-mediated recombination is the major route of evolution of degradative ability and PAH-degraders. Fourthly, even in those strains where genes for PAH degradation have been localized on chromosomes, clustering of the genes and flanking by transposon and sequence homology with plasmid borne counterparts, suggests acquisition by HGT and transposition. Finally, HGT and transposition seems to be more chronologically linked and less fortuitously directed than previously thought.

REFERENCES

Assinder SJ, Williams PA (1988). Comparison of the meta pathway operons on NAH plasmid pWW60-22 and TOL plasmid pWW53-4 and its evolutionary significance. J. Gen. Microbiol. 134: 2769-2778.

Atlas RM (1981). Microbial degradation of petroleum: an environmental perspective. Microbiol. Rev. 45:180-209.

Austen AA, Dunn NW (1980). Regulation of plasmid-specified naphthalene catabolic pathway of *Pseudomonas putida*. J. Gen. Microbiol., 117: 521-528.

Basta T, Keck A, Klein J, Stolz A (2004). Detection and characterization of conjugative degradative plasmids in xenobiotic-degrading Sphingomonas strains. J. Bacteriol., 186: 3862-3872.

Bosch R, Garcia-Valdes E, Moore ERB (1999). Genetic characterization and evolutionary implications of a chromosomally encoded naphthalene degradation upper pathway from *Pseudomonas stutzeri* AN10. Gene., 236: 149-157.

Bosch R, Garcia-Valdes E, Moore ERB (2000). Complete nucleotide sequence and evolutionary significance of a chromosomally encoded naphthalene degradation lower pathway from *Pseudomonas stutzeri* AN10. Gene., 245: 65-74.

Bosma TNP, Harms H, Zehnder AJB (2001). Biodegradation of xenobiotics in environment and technosphere. In: Beek B (ed) The Handbook of Environmental Chemistry Vol.2 Part K, Biodegradation and Persistence, Springer-Verlag, Berlin, Heidelberg, pp.163-202.

Broker D, Arenskotter M, Legatki A, Nies DH, Steinbuchel A (2004). Characterisation of a 101-kilobase-pair megaplasmid pKB1 isolated from the rubber-degrading bacterium *Gordonia westfalia* KB1. J. Bacteriol., 186: 212-225

Cane PA, Williams PA (1982). The plasmid-coded metabolism of naphthalene and 2-methylnaphthalene in *Pseudomonas* strains: phenotypic changes correlated with structural modification of the plasmid pWW60-1. J. Gen. Microbiol., 128: 2281-2290.

Cane PA, Williams PA (1986). A restriction map of naphthalene catabolic plasmid pWW60-1 and the location of some of its catabolic genes. J. Gen. Microbiol., l32: 2919-2929.

Cerniglia CE (1992). Biodegradation of polycyclic aromatic hydrocarbons. Biodegradation 3: 351-368.

Christie-Oleza JA, Lanfranconi MP, Nogaler B, Lalvcat J, Bosch R (2009). Conjugative interaction induces transposition of ISPs+p in *Pseudomonas stutzeri* AN10. J. Bacteriol., 191: 1239-1247.

Christie-Oleza JA, Nogales B, Martin – Cardona C, Lanfraconi MP, Alberti B, Lalucut J, Bosch R (2008). ISPst9, an ISL3-like insertion sequence from *Pseudomonas stutzeri* AN10 involved in catabolic inactivation. Int. Microbiol., 11: 101-110.

Connors MA, Barnsley EA (1982). Naphthalene plasmids in *Pseudomonas*. J. Bacteriol. 149: 1096-1101.

Coral G, Karagol S (2005). Isolation and characterisation of phenanthrene degrading bacteria from a petroleum refinerary soil. Ann. Microbiol., 55(4): 255-259.

Day MI (1982). Plasmids: studies in biology (No 142) Edward Arnold, London, p. 50.

Demaneche S, Meyer C, Micoud J, Louwagie , Willison, JC, Jouanneau Y (2004). Identification and functional analysis of two aromatic-ring-hydroxylating dioxygenases from a *Sphingomonas* strain that degrades various polycyclic aromatic hydrocarbons. Appl. Environ. Microbiol., 70: 6714-6725.

Dennis JJ, Zylstra GJ (2004). Complete sequence and genetic organization of pDTG1, the 83 kilobase naphthalene degradation plasmid from *Pseudomonas* putida strain NCIB 9816-4. J. Mol. Biol., 341: 753-768.

Denome SA, Stanley DC, Olson ES, Young KD (1993). Metabolism of dibenzothiophene and naphthalene in *Pseudomonas* strains: complete DNA sequence of an upper naphthalene catabolic pathway. J. Bacteriol., 175: 6890-6901.

Dunn NW, Dunn HM, Austen RA (1980). Evidence of two catabolic plasmids coding for the degradation of naphthalene. J. Gen.

Microbiol., 117: 521-528
Dunn NW, Gunsalus IC (1973). Transmissible plasmid coding early enzymes of naphthalene oxidation in *Pseudomonas* putida. J. Bacteriol., 114: 974-979.
Easter CL, Schwab H, Helinski DR (1998). Role of parCBA operon of the broad host range plasmid RK2 in stable plasmid maintenance. J. Bacteriol., 180: 6023-6030.
Eaton RW (1994). Organization and evolution of naphthalene catabolic pathways: sequence of the DNA encoding 2-hydroxychromene-2-carboxylate hydratase-aldolase from the NAH7 plasmid. J. Bacteriol., 176: 7757-7762.
Ensley BD, Ratzkin BJ, Osslund TD, Simon MJ, Wackett LP, Gibson DT (1983). Expression of naphthalene oxidation genes in Escherichia coli results in the biosynthesis of indigo. Sci., 222: 167-169.
Ferrell R (1979). Degradative plasmids; molecular nature and mode of evolution In: Timmis, K.N. and Puhler, A, plasmids of Medical, Environmental and Commercial Importance. Elsivier: North Holland. pp. 97-109.
Foght JM, Westlake DW (1996). Transposon and spontaneous deletion mutants of plasmid-borne genes encoding polycyclic aromatic degradation by a strain of *Pseudomonas* fluorescens. Biodegradation 7(4): 353-366.
Foster TJ (1983). Plasmid-determined resistance to antimicrobial drugs and toxic metals in bacteria. Microbiol. Rev. 47: 361-409.
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: 2522-2530.
Gerdes K, Moller-Jensen J, Ebersbach G, Kruse T, Nordstrom K (2004). Bacterial mitotic machineries. Cell, 116: 359-366.
Goyal AK, Zylstra GJ (1996). Molecular cloning of novel genes for polycyclic aromatic hydrocarbon degradation from Comamonas testosterone GZ39. Appl. Environ. Microbiol., 62: 230-236.
Goyal AK, Zylstra GJ (1997). Genetics of naphthalene and phenanthrene degradation by Comamonas testosteroni. J. Ind. Microbiol. Biotechnol., 19: 401-407.
Greated A, Lambertsen L, Williams PA, Thomas CM (2002). Complete sequence of the IncP-9 TOL plasmid pWWO from *Pseudomonas* putida. Environ. Microbiol., 4: 856-871.
Grimm AC, Harwood CS (1997). Chemotaxis of *Pseudomonas* sp. to the polyaromatic hydrocarbon naphthalene. Appl. Environ. Microbiol., 63: 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: 3310-3316.
Grund E, Denecke B, Eichenlaub R (1992). Naphthalene degradation via salicylate and genetisate by *Rhodococcus* sp. strain B4. Appl. Environ. Microbiol., 58:1874-1877.
Harayama S, Kok M, Niedle EL (1993). Functional and evolutionary relationships among diverse oxygenases. Annu. Rev. Microbiol., 46: 565-601.
Harayama S (1994). Codon usage patterns suggest independent evolution of two catabolic operons on toluene-degradative plasmid TOL pWWO of *Pseudomonas* putida. J. Mol. Evol., 38: 328-335.
Hardy K (1981). Bacterial plasmids (Aspects of microbiology). Thomas Nelson and Sons, Surrey. p. 1001.
Harwood CS, Parales RE (1996) The beta-ketoadipate pathway and the biology of self-identity. Annu. Rev. Microbiol. 50: 553-590.
Heitkamp MA, Franklin W, Cerniglia CE (1988). Microbial metabolism of polycyclic aromatic hydrocarbons: isolation and characterization of a pyrene degrading bacterium. Appl. Environ. Microbiol. 54: 2549-2555.
Herrick JB, Stuart-Keil KG, Ghiorse WC, Madsen EL (1997). Natural horizontal transfer of a naphthalene dioxygenase gene between bacteria native to a coal tar contaminated field site. Appl. Environ. Microbiol., 63: 2330- 2337.
Hwa KS, Jeong KH, Jin-cchul A, Hoon JJ, Seung-Yeol SL (2005). Characterisation of phenanthrene degradation by *Sphingomonas* sp. HS 362. Korean J. Microbiol., 41: 201-207.
Iwagami SG, Yang K, Davies J (2000). Characterization of the protocatechuic acid catabolic gene cluster from *Streptomyces* sp. strain 2065. Appl. Environ. Microbiol., 66: 1499-1508.
Jimenez IY, Bartha R (1996). Solvent-augmented mineralisation of pyrene by a *Mycobacterium* sp. Appl. Environ. Microbiol., 62: 2311-2316.
Johnsen AR, Wick LY, Harms H (2005). Principles of microbial PAH-degradation in soil. Environ. Poll., 133: 71-84.
Kanaly RA Harayama S (2010). Advances in the field of high-molecular-weight polycyclic aromatic hydrocarbon biodegradation by bacteria. Microbiol Biotechol., 3(2): 132-164.
Kanaly RA, Harayama S (2000). Biodegradation of high-molecular-weight polycyclic aromatic hydrocarbons by bacteria. J. Bacteriol., 182: 2059-2067.
Khan AA, Wang R-F, Cao W-W, Doerge DR, Wennerstrom D, Cerniglia CE (2001). Molecular cloning, nucleotide sequence, and expression of genes encoding a polycyclic aromatic ring dioxygenase from *Mycobacterium* sp. strain PYR-1. Appl. Environ. Microbiol., 67: 3577-3585.
Kim S-J, Kweon O, Jones RC, Freeman JP, Edmondson RD, Cerniglia, CE (2007). Complete and integrated pyrene degradation pathway in *Mycobacterium vanbaalenii* PYR-1 based on systems biology. J. Bacteriol., 189: 464-472.
Kiyohara H, Nagao K (1978). The catabolism of phenanthrene and naphthalene by bacteria. J. Gen. Microbiol., 105: 69-75.
Kiyohara H, Sugiyama M, Mondello FJ, Gibson DT, Yano K (1983). Plasmid involvement in the degradation of polycyclic aromatic hydrocarbons by *Beijerinckia* species. Biochem. Biophys. Res. Commun., 111: 939-945.
Kiyohara H, Takizawa N, Data H, Torigoe S, Yano K (1990). Characterization of a phenanthrene degradation plasmid from *Alcaligenes faecalis* AFK2. J. Ferment. Bioeng. 69: 54-56.
Kiyohara H, Torigoe S, Kaida N, Asaki T, Iida T, Hayashi H, Takizawa N (1994). Cloning and characterization of a chromosomal gene cluster, pah, that encodes the upper pathway for phenanthrene and naphthalene utilization by *Pseudomonas putida* OUS82. J. Bacteriol., 176: 2439-2443.
Komatsu T, Omori T, Kodama T (1993). Microbial degradation of the polycyclic aromatic hydrocarbons acenaphthene and acenaphthylene by a pure bacterial culture. Biosci. Biotechnol. Biochem., 57: 864-865.
Konig C, Eulberg D, Groning J, Lakner S, Seibert V, Kaschabek SR, Schlomann M (2004). A linear megaplasmid, p1CP, carrying the genes for chlorocatechol catabolism of *Rhodococcus opacus* 1CP. Microbiol., 150: 3075-3087.
Kouzuma A, Pinyankong O, Nojiri H, Omori T, Yamane H, Habe H (2006). Functional and transcriptional analyses of the initial oxygenase genes for acenaphthene degradation from *Sphingomonas* sp. strain A4. Microbiol., 152: 2455-2467.
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: 3828-3841.
Kumar G, Singlar, Kumar R (2010). plasmid associated anthracene degradation by *Pseudomonas* sp. isolated from filling station site. Nature Sci., 8(4): 89-94.
Kurkela S, Lehvaslaiho H, Palva ET, Teeri TH (1988). Cloning, nucleotide sequence, and characterization of genes encoding naphthalene dioxygenase of *Pseudomonas putida* strain NCIB9816. Gene., 73: 355-362.
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: 531-540.
Lawrence JG, Ochman H (2002). Reconciling the many faces of lateral gene transfer. Trends Microbiol., 10: 1-4.
Leahy JG, Colwell RR (1990). Microbial degradation of hydrocarbons in the environment. Microbiol. Rev., 54: 305-315.
Lily MK, Bahuguna A, Dangwal K, Garg V (2010). Optimisation of inducible, chromosomally encoded benzo(a)pyrene degradation pathway in *Bacillus subtilis* BMT4i (MTCC 9447). Ann. Microbiol., 60: 51-58.
Lin Y, Cai L-X (2008). PAH-degrading microbial consortium and its pyrene-degrading plasmids from mangrove sediment samples in Huian, China. Mar. Pollut. Bull., 57: 703-706.
Ma Y, Wang L, Shao Z (2006). *Pseudomonas*, the dominant polycyclic aromatic hydrocarbon-degrading bacteria isolated from Antarctic soils and the role of large plasmids in horizontal gene transfer. Environ.

Microbiol., 8(3): 455-465.
Mallick S, Chatterjee S, Dutta TK (2007). A novel degradation pathway in the assimilation of phenanthrene by Staphylococcus sp. strain PN/Y via meta-cleavage of 2-hydroxy-1-naphthoic acid: formation of trans-2,3-dioxo-5-(29-hydroxyphenyl)-pent-4-enoic acid. Microbiol., 153: 2104-1215.
Miller CD, Child R, Hughes JE, Benscai M, Dar JP, Sims RC, Anderson AJ (2007). Diversity of soil *Mycobacterium* isolates from three sites that degrade polycyclic aromatic hydrocarbons. J. Appl. MIcrobiol., 102:1612-1624.
Mastral AM, Callén M (2000). A review on polycyclic aromatic hydrocarbon (PAH) emissions from energy generation. Environ. Sci. Technol., 34: 3051-3057.
Moody JD, Freeman JP, Fu PP, Cerniglia CE (2004). Degradation of benzo[a]pyrene by *Mycobacterium vanbaalenii* PYR-1. Appl. Environ. Microbiol., 70: 340-345.
Mrozik A, Piotrowska-Seget B, Labuzek S (2003). Bacterial degradation and bioremediation of polycyclic aromatic hydrocarbons. Polish J. Environ. Stud., 12(1): 15-25.
Neher TM, Lueking DR (2009). Naphthalene assimilation in *Pseudomonas* fluorescens: plasmid localization of gene for OmpW. Canadaian J. Microbiol., 55(5): 553-563.
Obayori OS, Ilori MO, Adebusoye SA, Oyetibo GO, Amund, OO (2008). Pyrene-degradation potentials of *Pseudomonas* species isolated from polluted tropical soils. World J. Microbiol. Biotechnol., 24: 2639-2646.
Okoro CC, Amund OO, Eliora ER (2009). Plasmids of phenanthrene and dibenzothiophene degrading bacteria isolated from produced water samples of oil production operations. Int. J. Chem. Biol. Sci., 3(2): 186-191.
Okpokwasili GC, Somerville CC, Grimes DJ, Colwell RR (1984). Plasmid-associated phenanthrene degradation by Chesapeake Bay sediment bacteria. Colloq. Inst. Fr. Rech. Explort. Mar., 3: 601-610.
Parales RE, Bruce NC, Schmid A, Wackett LP (2002). Biodegradation, biotransformation, and biocatalysis (B3). Appl. Environ. Microbiol., 68: 4699-4709.
Park W, Jeon CO, HohnStock-Ashe AM, Winans SC, Zylsra GJ, Eugene L (2003). Identification and characterization of the conjugal transfer region of the pCg1 plasmid from naphthalene-degrading *Pseudomonas putida* Cg1. Appl. Environ. Microbiol., 69: 3263-3271.
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. Microbiol., 148: 2319-2329.
Pinyakong O, Habe H, Kouzuma A, Nojiri H, Yamane H, Omori T (2004). Isolation and characterization of genes encoding polycyclic aromatic hydrocarbon dioxygenase from acenaphthene and acenaphthylene degrading *Sphingomonas* sp. strain A4. FEMS Microbiol. Lett., 238: 297-305.
Pinyakong O, Habe H, Yoshida T, Nojiri H, Omori T (2003b). Identification of three novel salicylate 1-hydroxylases involved in the phenanthrene degradation of *Sphingobium* sp. strain P2. Biochem. Biophys. Res. Commun., 301: 350-357.
Platt A, Shingler V, Taylor SC, Williams PA (1995). The 4-hydroxy- 2-oxovalerate aldolase and acetaldehyde dehydrogenase (acylating) encoded by the nahM and nahO genes of the naphthalene catabolic plasmid pWW60–22 provide further evidence of conservation of meta-cleavage pathway gene sequences. Microbiol., 141: 2223-2233.
Rentz JA, Alvarez PJJ, Schnoor JL (2005). Benzo[a]pyrene co-metabolism in the presence of plant root extracts and exudates: implications for phytoremediation. Environ. Pollut., 136: 477-484.
Rentz JA, Alvarez PJJ, Schnoor JL (2008). Benzo[a]pyrene degradation by *Sphingomonas yanoikuyae* JAR02. Environ. Pollut., 151: 669-677.
Romine MF, Stillwell LC, Wong K-K, Thurston SJ, Sisk EC, Sensen C, Gaasterland T, Fredrickson JK, Saffer JD (1999). Complete sequence of a 184-kilobase catabolic plasmid from *Sphingomonas aromaticivorans* F199. J. Bacteriol., 181: 1585-1602.
Rossello-Mora RA, Lalucat J, García-Valdez E (1994). Comparative biochemical and genetic analysis of naphthalene degradation among *Pseudomonas stutzeri* strains. Appl. Environ. Microbiol,. 60: 966-972.
Saito A, Iwabuchi T, Harayama S (2000). A novel phenanthrene dioxygenase from Nocardioides sp. strain KP7: expression in *Escherichia coli*. J. Bacteriol., 182: 2134-2141.
Samanta SK, Jain RK (2000). Evidence for plasmid-mediated chemotaxis of *Pseudomonas putida* towards naphthalene and salicylate. Can. J. Microbiol., 46: 1-6.
Sanseverino J, Applegate BM, King JMH, Sayler GS (1993). Plasmid-mediated mineralization of naphthalene, phenanthrene, and anthracene. Appl. Environ. Microbiol., 59: 1931-1937.
Schell MA (1983). Cloning and expression in Escherichia coli of the naphthalene degradation genes from plasmid NAH7. J. Bacteriol., 153: 822-829.
Schell MA, Wender PE (1986). Identification of the nahR gene product and nucleotide sequence required for its activation of the sal operon. J. Bacteriol., 166: 9-14.
Schneider J, Grosser R, Jayasimhulu K, Xue W, WarshaD (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. Microbio. 62: 13-19.
Selifonov SA, Slepen'kin AV, Adanin VM, Grechkina GM, Starovoitov II (1993). Acenaphthene catabolism by strains of *Alcaligenes eutrophus* and Alcaligenes paradoxus. Microbiol., 62: 85-92.
Sentchillo VSPA, Zehnder AJB, van der Meer JR (2000). Molecular diversity of plasmids bearing genes that encode toluene and xylene metabolism in *Pseudomonas* strains isolated from different contaminated sites in Belarus. Appl. Environ. Microbiol., 66: 2842-2852.
Sevastsyanovich YR, Krasowiak R, Bingle LEH, Haines AS, Sokolov SL, Kosheleva IA, Leuchuk AA, Titok MA, Smalla K, Thomas CM (2008). Diversity of IncP-9 plasmids of *Pseudomonas*. Microbiol., 154: 2929-2941.
Shi T, Fredrickson JK, Balkwill DL (2001). Biodegradation of polycyclic aromatic hydrocarbons by *Sphingomonas* strains isolated from the terrestrial subsurface. J. Ind, Microbiol. Biotechnol., 26: 283-289.
Shimizu S, Kobayashi H, Masai E, Fukuba M (2001). Characterisation of the 450-kb linear plasmid in a polychlorinated biphenyl degrader *Rhodococcus* sp. strain RHA1. Appl. Environ. Microbiol., 67: 2021-2028.
Sho M, Hamel C, Greer CW (2004). Two distinct gene clusters encode pyrene degradation in Mycobacterium sp. strain S65. FEMS Microbiol. Ecol., 48: 209-220.
Simon MJ, Osslund TD, Saunders R, Ensley BD, Suggs S, Harcourt A, Suen W-C, Cruden DL, Gibson DT, Zylstra GJ (1993). Sequence of genes encoding naphthalene dioxygenase in *Pseudomonas putida* strains G7 and NCIB 9816–4. Gene., 127: 31-37.
Sorensen SJ, Bailey M, Hansen LH, Kroer N, Wuertz S (2005). Studying plasmid horizontal transfer in situ: a critical review. Nature, 3: 700-710.
Stecker C, Johann A, Herzberg C, Averhoff B, Gottschalk G (2003). Complete nucleotide sequence and genetic organisation of the 210-kilobase linear plasmid of *Rhodococcus erythropolis* BDZ. J. Bacteriol., 185: 5269-5274.
Stuart-Keil KG, Hohnstock AM, Drees KP, Herrick JB, Madsen EL (1998). Plasmids responsible for horizontal transfer of naphthalene catabolism genes between bacteria at a coal tar-contaminated site are homologous to pDTG1 from *Pseudomonas putida* NCIB 9816–4. Appl. Environ. Microbiol., 64: 3633-3640.
Takizawa N, Kaida N, Torigoe S, Moritani T, Sawada T, Satoh S, Kiyohara H (1994). Identification and characterization of genes encoding polycyclic aromatic hydrocarbon dioxygenase and polycyclic aromatic hydrocarbon dihydrodiol dehydrogenase in *Pseudomonas putida* OUS82. J. Bacteriol., 176: 2444-2449.
Top EM, Springael D (2003). The role of mobile genetic elements in bacterial adaptation to xenobiotic organic compounds. Curr. Opin. Biotechnol., 14: 262-269.
Top EM, Springael D, Boon N (2002). Catabolic mobile genetic elements and their potential use in bioaugmentation of polluted soils and waters. FEMS Microbiol. Ecol., 42: 199-208.
Tsuda M, Iino T (1990). Naphthalene degrading genes on plasmid NAH7 are on a defective transposon. Mol. Gen. Genet., 223: 33-39.
Van der Meer JR, DE VOS WM, Harayama S, Zehnder AJB (1992). Molecular mechanisms of genetic adaptation to xenobiotic compounds. Microbiol. Rev., 56: 677-694.

Van Melderen L (2002). Molecular interactions of the CcdB poison with its bacterial target, the DNA gyrase. Int. J. Med. Microbiol., 291: 537-544.
Wackett LP, Hershberger LCD (2001). Biocatalysis and biodegradation: microbial transformation of organic compounds. ASM Press, Washington.
Wheelis M (1975). The genetics of dissimilatory pathways in *Pseudomonas*. Annu. Rev. Microbiol., 29: 505-524.
Williams PA, Murray K (1974). Metabolism of benzoate and the methylbenzoates by *Pseudomonas putida* (arvilla) mt-2: evidence of the existence of TOL plasmid. J. Bacteriol., 120: 416-423.
Wilson MS, Herrick JB, Jeon CO, Hinman DE, Madsen EL (2003). Horizontal transfer of phnAc dioxygenase genes within one of two phenotypically and genotypically distinctive naphthalene degrading guilds from adjacent soil environments. Appl. Environ. Microbiol., 69: 2172-2181.
Yen K-M, Gunsalus IC (1982). Plasmid gene organization: naphthalene/salicylate oxidation. Proc. Natl. Acad. Sci. USA, 79: 874-878.
Yen K-M, Gunsalus IC (1985). Regulation of naphthalene catabolic genes of plasmid NAH7. J. Bacteriol., 162: 1008-1013.
Yen K-M, Serdar CM (1988). Genetics of naphthalene catabolism in pseudomonads. CRC Crit. Rev. Microbiol., 15: 247-268.
You IS, Ghosal D, Gunsalus IC (1991). Nucleotide sequence analysis of the Pseudomonas putida PpG67 salicyclate gene (nahG0 and its 3'-flanking region. Biochemistry, 30(6): 1635-1641.