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Review

Microbial transformation of xenobiotics for environmental bioremediation

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The accumulation of recalcitrant xenobiotic compounds is due to continuous efflux from population and industrial inputs that have created a serious impact on the pristine nature of our environment. Apart from this, these compounds are mostly carcinogenic, posing health hazards which persist over a long period of time. Metabolic pathways and specific operon systems have been found in diverse but limited groups of microbes that are responsible for the transformation of xenobiotic compounds. Distinct catabolic genes are either present on mobile genetic elements, such as transposons and plasmids, or the chromosome itself that facilitates horizontal gene transfer and enhances the rapid microbial transformation of toxic xenobiotic compounds. Biotransformation of xenobiotic compounds in natural environment has been studied to understand the microbial ecology, physiology and evolution for their potential in bioremediation. Recent advance in the molecular techniques including DNA fingerprinting, microarrays and metagenomics is being used to augment the transformation of xenobiotic compounds. The present day understandings of aerobic, anaerobic and reductive biotransformation by co-metabolic processes and an overview of latest developments in monitoring the catabolic genes of xenobiotic-degrading bacteria are discussed elaborately in this work. Till date, several reviews have come up, highlighting the problem of xenobiotic pollution, yet a comprehensive understanding of the microbial biodegradation of xenobiotics and its application is in nascent stage. Therefore, this is an attempt to understand the microbial role in biotransformation of xenobiotic compounds in context to the modern day biotechnology.

Key words: Xenobiotics, degradation, bioremediation, co-metabolism, catabolic genes, plasmid, transposon, horizontal gene transfer.

INTRODUCTION

Both natural and anthropogenic activities result in accumulation of wide ranges of toxic xenobiotic compounds in the environment, and thus cause a global concern (Gienfrada and Rao, 2008). Primarily, xenobiotics are those compounds that are alien to a living individual and have a propensity to accumulate in the environment. Principal xenobiotics include pesticides, fuels, solvents, alkanes, polycyclic hydrocarbons (PAHs), antibiotics, synthetic azo dyes, pollutants (dioxins and polychlorinated biphenyls), polyaromatic, chlorinated and nitro-aromatic compounds. The main concern with xenobiotic compounds is the toxicity threat they pose to public health. It is quite shocking that some xenobiotic compounds (phenols, biphenyl compounds, phthalates, etc.) act as endocrine disruptors (Nagao, 1998; Borgeest et al., 2002).

Biodegradation is one of the natural processes that help to remove xenobiotic chemicals from the environment by microorganisms. This is primarily a strategy for the survival of the microorganisms (Singh, 2008). It is one of the most cost-effective methods amongst remedial approaches. Several excellent reviews have been published on the biodegradation or bioremediation, both generally (Prescott et al., 2008; Chatterjee et al., 2008)

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or specifically, of xenobiotic compounds (Austin et al., 1977; Chaudhry and Chapalamadugu, 1991; Zhang and Bennet, 2005; Chauhan et al., 2008; Chowdhury et al., 2008). Although most organisms have detoxifying abilities (that is, mineralization, transformation and or immobilization of pollutants), microorganisms, particularly bacteria, play a crucial role in biogeochemical cycles for sustainable development of the biosphere (Tropel and Van der Meer, 2004). Horizontal gene transfer, high growth rates and metabolic versatility make them to evolve quickly and to adapt themselves to changing conditions of environment, even at extreme environment that does not permit the proliferation of other living organisms (Timmis and Pieper, 1999; Diaz and Prieto, 2000; Kim and Crowley, 2007). A large number of microbial communities have been characterized with their responses to pollutants, in order to identify the potential bacterial degrader that can adapt to use these chemicals as their novel growth and energy substrates (Janssen et al., 1985).

Microbial degradation of petroleum and other hydrocarbons is incredibly an intricate course of action that mainly depends on the composition of community and its adaptive response to the presence of these compounds. Such degradation of petroleum in marine sites is restricted principally by the availability of phosphorus and nitrogen. Apart from this pH, moisture, oxygen and temperature are prime factors influencing the degradation rate (Leahy and Colwell, 1990). The biological treatment of xenobiotic wastes in the conventional activated sludge process varies under different conditions (Widada et al., 2002). Relevance of current progress in molecular techniques has to be identified for isolation of plasmid DNA, construction of DNA probes in recent perspective to explore the efficient genes implicated in catabolism of xenobiotics and to study the genetic diversity of environmentally significant microbes.

The intent of the present review paper is to present a broad and updated overview of current understanding of the physiological, genetical and the molecular approaches for biodegradation of certain xenobiotic compounds by microorganisms.

ROLE OF MICROBES

Pollution of soil and water through xenobiotics is an intense dilemma (Moriarty, 1988). Microorganisms represent half of the biomass of our planet, yet we know as little as 5% of the microbial diversity of the biosphere (Curtis and Reinhard, 1994). However, the great versatility of microbes offers a simpler, economical and more environmental friendly strategy to reduce environmental pollution and to help in biodegradation of xenobiotic compounds. In addition, they play a crucial role in biogeochemical cycles and in sustainable development of the biosphere. The bacterial genera found to undergo bio-fix, a wide range of xenobiotic chemicals include both aerobic e.g. *Pseudomonas*, *Escherichia*, *Sphingobium*, *Pandoraea*, *Rhodococcus*, *Gordonia*, *Bacillus*, *Moraxella*, *Micrococcus* and anaerobic types e.g. *Pelatomaculum*, *Desulfotomaculum*, *Syntrophobacter*, *Syntrophus*, *Desulphovibrio*, *Methanospirillum*, *Methanosaeta* (Chowdhury et al., 2008). It is found that *Pseudomonas* BCb12/1 and BCb12/3 are of excellent degradation capabilities of low ethoxylated NP*n*EO (Non phenol Polyethoxylates), maintaining high cell vitality (DiGioia et al., 2008). Several other bacterial genera that help in the degradation of toxic and recalcitrant xenobiotic compounds are listed in Table 1.

Since bacterial genera aid efficiently in biotransformation processes, evaluation of the xenobiotic contaminated areas is quite essential and involves the enumeration and detection techniques of the xenobiotic degraders that generate quick and safe consequences. The enumeration as well as monitoring of xenobiotic-degrading bacterial populations in contaminated environment with traditional microbiological methods can acquire an inordinate length of time (Lloyd-Jones et al., 1999). To meet this challenge, currently molecular approaches are practiced to exemplify the nucleic acids of microorganisms contained in the microbial community from environmental samples (Widada et al., 2002). In spite of this, when molecular techniques are combined with basic microbiological methods, a comprehensive interpretation of the *in situ* microbial community along with its response to both engineered bioremediation and natural attenuation processes is attained (Brockman, 1995).

The *in-situ* bioremediation process consists of three fundamental steps (Madsen, 1991):

(i) Bioattenuation: monitoring natural progress of degradation to ensure that contaminant decreases with time of sampling.
(ii) Biostimulation: intentional stimulation of resident xenobiotic-degrading bacteria by electron acceptors, water, nutrient addition, or electron donors.
(iii) Bioaugmentation: addition of laboratory grown bacteria that have appropriate degradative abilities.

Bacteria have developed strategies for obtaining energy from virtually every compound under oxic or anoxic conditions universally by using ultimate electron acceptors such as nitrate, sulfate and ferric ions. Amongst xenobiotic compounds, benzene ring is next to glucosyl residues and most extensively found unit of chemical structure in nature (Diaz, 2004). Either aerobically or anaerobically, the bacterial enzymes are highly efficient to break resonance structure and to help in operating carbon cycle. Gene clusters that code for the catabolism of aromatic compounds are frequently found in mobile genetic elements, as transposons and plasmids, which assist their horizontal gene transfer, and consequently enhance adaptation of specific bacterial genera to novel

Table 1. List of xenobiotic compounds and degrading bacterial genera.

Target compounds	Bacteria degrading the compounds	References
Pesticides		
Endosulfan compounds	*Mycobacterium* sp.	Sutherland et al., 2002
Endosulphate compounds	*Arthrobacter* sp.	Weir et al., 2006
HCH	*Pseudomonas putida*	Benezet and Matusumura, 1973
2,4-D	*Alcaligenes eutrophus*	Don and Pemberton, 1981
DDT	*Dehalospirilum multivorans*	Chaudhry and Chapalamadugu, 1991
Halogenated organic compounds		
Vinylchloride .	*Dehalococcoides* sp.	He et al., 2003
Atrazine .	*Pseudomonas* sp.	Bruhn et al., 1988
PCE	*Dehalococcoides ethenogenes*195	Magnuson et al., 2000
PAH compounds		
Napthalene	*Pseudomonas putida*	Habe and Omori, 2003
PCP	*Psedomonas* sp.	Yen and Serdar, 1988
3CBA	*Arthrobacter* sp.	Pignatello et al., 1983
1,4DCB	*Alcaligenes* sp.	Don and Pemberton, 1981
2,3,4-chloroaniline	*Pseudomonas* sp.	Spain and Nishino, 1987
2,4,5-T	*Pseudomonas* sp.	Latorre et al., 1984
Fluoranthrene	*Pseudomonas cepacia* AC1100	Karns et al., 1983
Pyrene	*Mycobacterium*PYR-1	Kanaly and Harayama, 2000
	Sphingomonas paucimobilis	Habe and Omori, 2003
Phthalate compounds		
Phthalate	*Burkholderiacepacia*DBO1	Chang and Zylstra, 1999
Other compounds		
PCB	*Rhodococcus*RHA1	Kimbara, 2005
Dioxins	*Dehalococcoides* sp.	Bunge et al., 2003
RDX	*Desulfovibrio* sp.	Boopathy and Kulpa, 1998
Benzene	*Dechloromonas* sp.	Coates et al., 2001
Petroleum products		
	Achromobacter sp. *Acinetobacter* sp. *Micrococcus* sp. *Nocardia* sp. *Bacillus* sp. *Flavobacterium* sp.	Austin et al., 1977
Azo dyes	*Bacillus* sp. *Pseudomonas* sp. *Sphingomonas* sp. *Xanthomonas* sp.	Dykes et al., 1994 Stolz, 2001 Stolz, 2001 Reife and Freeman, 2000

pollutants.

BIOCHEMICAL PATHWAYS

The pathways of degradation are mainly of two broad options, either aerobic or anaerobic. Wide phylogenetic diversities of bacteria are capable of degrading contaminants aerobically; they degrade PCB (Poly-chlorinated Biphenyls) very efficiently e.g: *Pseudomonas*, *Bacillus* JF8 (Kimbara, 2005). As anoxic conditions mostly prevail in the polluted sites, several bacterial species that are anaerobic or facultative aerobic use natural organics as carbon and energy sources e.g. methanogens, denitrifying microbes, reducers [Mn, Cr, Fe (III), SO_4^{3-}, ClO^{4-}] etc. (Lovley, 2003). Few xenobiotic

compounds of environmental concern have naturally occurring relatives and their repeated exposure has resulted in the adaptation and evolution of anaerobic bacteria capable of metabolizing these man-made compounds (Zhang and Bennet, 2005). Co-metabolism is another exclusive phenomenon; it appears to occur widely in microbial metabolism. Here, the micro-organisms transform the desired xenobiotic compound even though the compound itself cannot serve as the primary energy source for those organisms. To degrade the contaminant, the microbes require the presence of other compounds (primary substrates) that can support their growth. The enzymes or coenzymes produced to degrade the primary substrate may display some activity for other substrate which is significantly known as co-substrate. The co-substrate is not the physiologically intended substrate but is just 'accidentally' transformed or in other words, the process occurs in a much randomized manner (Horvath, 1972).

Aerobic pathway

In response to immense turnover of the aromatic compounds in the carbon cycle, well organized channels of aerobic catabolism have been evolved separately with evolution (Chauhan et al., 2008). The aerobic strategy of degradation shows advantages over anaerobic ones. Commonly, the aerobic organisms overcome the problem of degradation with oxygenases that initially reduce elemental oxygen to activate it, permitting it to insert into inert xenobiotic molecule. Whereas, under anaerobic conditions oxygen is inserted by some other source that is, organic acids are added onto hydrocarbons (of xenobiotic molecule) with synthases; oxygen in water is inserted into double bonds with hydratases and carbonic acid is added onto molecules with carboxylases. While compared with oxygen, most of the alternative electron acceptors have lower standard reduction potential ($E°'$) values and convert into less standard Gibbs free energy change ($\Delta G°'$) when coupled to the oxidation of any given substrate. Thus it appears that aerobic culture techniques are relatively simple, as well as efficient and generally applicable through oxidative degradation (Adriaens and Vogel, 1995).

The aerobic catabolic funnel mostly includes peripheral pathways involving oxygenation reactions carried out by monooxygenases or hydroxylating dioxygenases and generates dihydroxy aromatic compounds. These intermediate compounds are processed through either ortho or meta cleavage leading to central intermediates such as protocatechuates, catechols, gentisates, homoprotocatechuates, homogentisates, hydroquinones and hydroxyquinols, which are further transformed to tricar-boxylic acid cycle intermediates (Harayama and Timmis, 1992) and finally channeled into the intermediates of Kreb's cycle.

However, in case of dichlorodiphenyltrichloroethane (DDT), pentachlorophenol (PCP) and 1,2,3,4,5,6-hexachlorocyclohexane (HCH) mode of aerobic ring cleavage is different. They are initially reduced to less chlorinated intermediates and finally undergo enzymatic transformations by microbes (Mishra et al., 2001). Despite the evolutionary adaptation of the aromatic ring degrading enzymes for specific substrates, the enzymes for a particular pathway can catalyze the transformation of a range of aromatic compounds, showing less specificity (Copley, 2000). Bacterial dehalogenases catalyze the cleavage of carbon-halogen bonds, which is a key step in aerobic mineralization pathways of several halogenated pollutants (Janssen et al., 2005) (Figure 1).

Most common aerobic pathway for alkane degradation is oxidation of the terminal methyl group into a carboxylic acid through an alcohol intermediate and eventually completes mineralization through beta-oxidation (Leahy and Colwell, 1990). The degradation of phthalate isomers begin initially with esterases followed by permeases and finally by dioxygenases (Vamsee and Phale, 2008). 3,4-dihydroxybenzoate acts as central intermediate in most cases and undergoes several gradations resulting in acetyl-CoA and succinyl-CoA as end products. Bacteria play major role on or after the degradation of 3,4-dihydroxybenzoate, for example: *Rhodococcus* RHA1 and *Arthrobacter keyseri* 12B (Eaton, 2001; Hara et al., 2007).

Strains of *Pseudomonas, Acetobacter and Klebsiella* have the ability to grow with carcinogenic azo compounds as well as exclusively produce true azo reductases, which reductively cleave 4'dimethylaminoazobenzene 2 carboxylic acid [commonly known as acid red 2 (methyl red)]. The reductive decolourization of sulfonated azo dyes such as acid orange 7, acid orange 10, acid red 88, acid red 4 and acid orange 8 are efficiently accomplished by miscellaneous strains such as *Pseudomonas, Sphingomonas, Xanthomonas, Aeromonas* and *Bacillus* during aerobic conditions in the presence of additional carbon sources (Stolz, 2001). Bacteria that are able to reduce azo dyes aerobically in a co-metabolic pathway can also use these dyes as sole source of carbon and energy (Yatome et al., 1993; Dykes et al., 1994; Stolz, 2001). However, there are very few studies that unequivocally demonstrate the utilization of azo compounds as sole source of carbon and energy under aerobic conditions.

Anaerobic pathway

The degradation of xenobiotic compounds by anaerobic microbes (e.g. *Clostridia*, *Desulfobacterium*, *Desulfovibrio*, *Methanococcus*, *Methanosarcina* and dehalogenating bacteria) has been a subject of extensive research during the last two decades (Gibson and Harwood, 2002). In the absence of molecular O_2, alternative

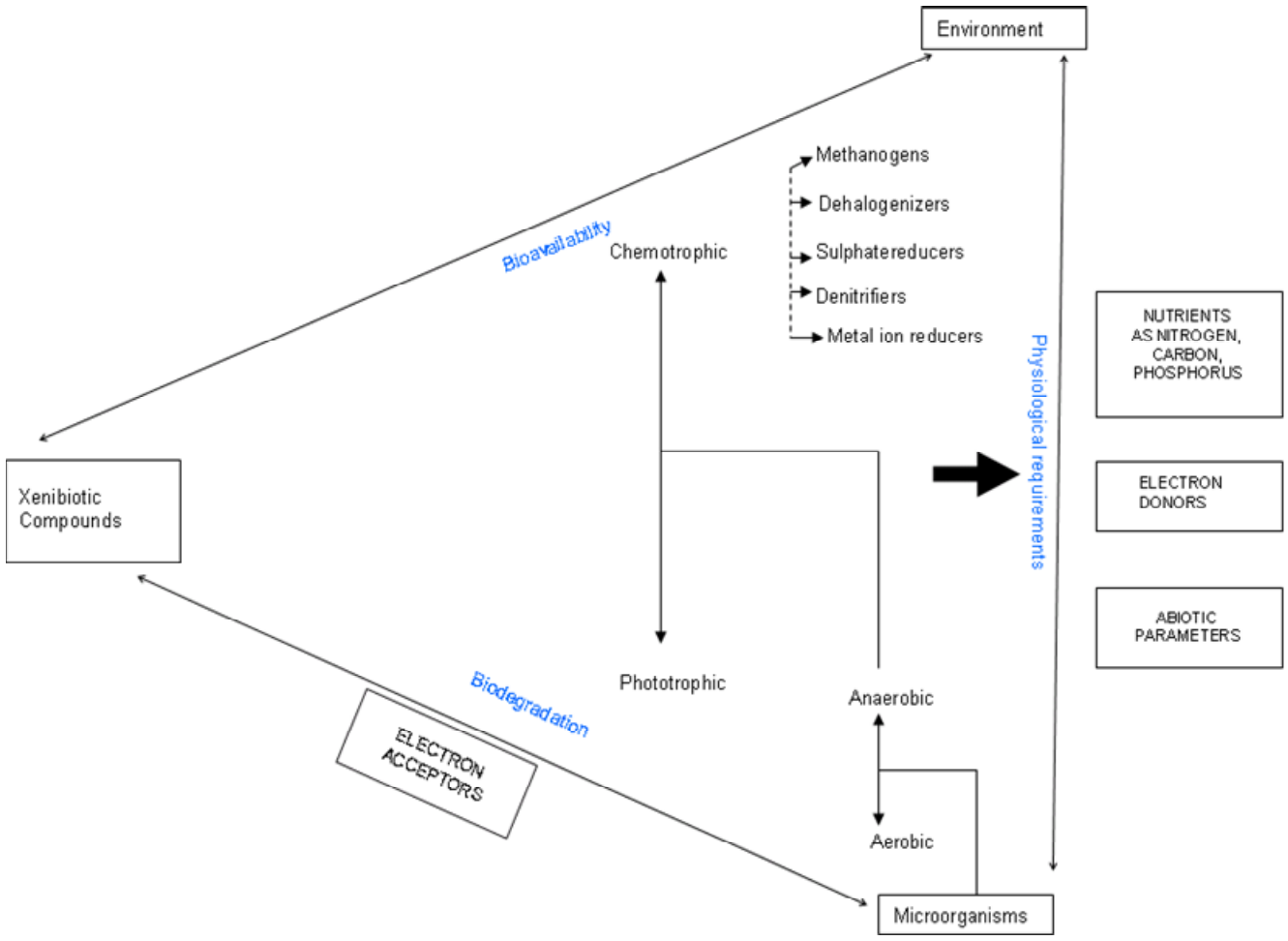


Figure 1. Interactions within ecosystem where microbes, environment and xenobiotic compounds are three limiting factors.

used to oxidize aromatic compounds (Zhang and Bennet, 2005; Chauhan et al., 2008). Xenobiotic compounds are oxidized and mineralized under anaerobic conditions if they are able to serve as electron donating substrates of primary metabolism. Phenols, phthalates and hydrocarbons including benzene-toluene-ethyl benzene-xylene (BTEX) fall into this category (Reuter et al., 1994). Several xenobiotic compounds themselves act as terminal electron acceptors (TEA), supporting growth of microorganisms by gaining energy from the oxidation of simple substrates (e.g. H_2).

Pesticides, during anaerobic degradation, undergo dechlorination, hydrolysis, nitro reduction and dealkylation, which generally has a metabolic shift from one pathway to another. Reductive dechlorination is common to all halogenated pesticides including aliphatic, cyclic aliphatic, aromatic, aniline-based phenoxyalkanotes and cyclodiene types as the terminal electron accepting the process. This metabolism is referred to as halorespiration or dehalorespiration (Cutter et al., 2001). Reductive dehalogenases constitute a novel pathway intended for complete dechlorination of tetrachloroethylene or perchloroethylene (PCE) to ethene, shown in Figure 2a, is obtained from an anaerobic microbial enrichment culture containing *Dehalococcoides ethenogenes* 195 (Magnuson et al., 2000).

The use of trinitrotoluene (TNT) as a terminal electron acceptor linked to growth has also been claimed (Esteve-Nunez et al., 2000). Anaerobic transformation of TNT at low redox potential minimizes oxidative polymerization and releases toxic azoxy compounds that form readily in the presence of oxygen. However, the enzymes have not been characterized completely (Mishra et al., 2001; Esteve-Nunez et al., 2001). Xenobiotics with electron-withdrawing substituents as azo dyes, cyclotrimethylenetrinitramine (RDX) and carbon tetrachloride are readily biotransformed by cometabolic reduction processes (Janssen et al., 2005). Benzoyl coenzyme-A (CoA) pathway is one of the centralized pathways in majority of denitrifying anaerobes where key enzyme is benzoate coenzyme A ligase which attaches the coenzyme to the carboxy group and activates the benzoate for metabolism. Oxygen is incorporated and a subsequent hydratase breaks the ring open. Thereafter, a series of

reaction steps precede chopping away at simple aliphatic acids until acetyl-CoA is formed (Schink et al., 2000). A large majority of monocyclic aromatic compounds funnel into the benzoyl-CoA pathway as shown in Figure 2b.

Phthalate compound degradation is mainly carried out by anaerobic methanogens (*Methanospirillum hungatei, Methanosaeta concilii, Syntrophobacter fumaroxidens*), producing acetate and methane as end products by decarboxylation initially, then reduction followed by ring cleave and ultimately pave to the β-oxidation pathway (Qiu et al., 2004; Zhang and Bennet, 2005). BTEX compounds act as carbon and energy sources for diverse anaerobic bacteria under nitrate-reducing, Fe (III) reducing, sulphate-reducing and methanogenic conditions. Methyl tert-butyl ether (MTBE), a parallel contaminant with BTEX and PAHs in petroleum-contaminated sites, prone to aerobic degradation generally, and also on the anaerobic metabolism of MTBE, has been reported (Kolhatkar et al., 2002). Truly with an anaerobe, the cleavage involves methyl transferases with tetrahydrofolate for the degradation of lignin and hydroxyl group addition during fermentation of polyethylene glycols but with an aerobic, bacteria degradation occurs with mono oxygenase (Zhang and Bennet, 2005).

The biotransformation of pyrene and benzo[a]pyrene (BaP) is well studied in different bacterial species such as *Mycobacterium vanbaalenii* PYR-1, *M. flavescens* PYR-GCK, *Mycobacterium* RJGII-135, *Mycobacterium* KR2 and *Mycobacterium*AP1. Different pathways have been anticipated. *Mycobacterium* KMS has been used to study the metabolites produced during pyrene transformation. First pathway indicates that pyrene hydroxylation takes place at the 1,2 positions, leading to the formation of 4-hydroxy-perinaphthenone which is the ultimate product so far found only in *M. vanbaalenii* PYR-1 cultures (Khan et al., 2001). Another pathway involves the accumulation of 6,6′-dihydroxy-2,2′-biphenyl-dicarboxylic acid in *Mycobacterium* sp. strain AP1. Several key metabolites, including pyrene-4,5-dione, *cis*-4,5-pyrene-dihydrodiol, phenanthrene-4,5-dicarboxylic acid, and 4-phenanthroic acid are obtained. Pyrene-4,5-dione accumulates as a final product in some gram-negative bacteria, and is further utilized followed by biotransformation (Liang et al., 2006). The generalized pathway of biodegradation of pyrene and BaP is presented in Figure 2c. Pyrene-4,5-dione can be formed following the auto oxidation of 4,5-dihydroxy-pyrene and is also a pyrene transformation metabolite in several bacteria as in *Sphingomonas yanoikuyae* R1 (Khan et al., 2001).

Co-metabolic pathway

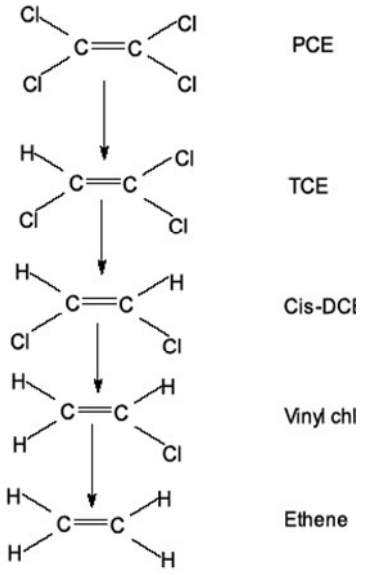
The oxidation of ethane to acetic acid, propane to propanoic acid and butane to butanoic acid and methyl ethyl ketone during growth of *Pseudomonas methanica* on methane initiated the concept of co-metabolism (Jensen, 1963). Though co-metabolism entails the parallel oxidation of a non-growth substrate during growth of bacteria on a compatible carbon and energy source, it also describes oxidation of non-utilizable substrates by resting cell suspensions grown at the disbursement of substances that are capable of supporting microbial growth. Consequently, practice of co-metabolism refers to oxidation of substances without utilization of the energy derived from the oxidation to support microbial growth and does not infer presence or absence of growth substrate during the oxidation. So, it is a technique generally employed for the biochemical study of microbial aromatic metabolism (Horvath, 1972).

Due to rapid decomposition of the herbicide, dalapon is observed without an increase in bacterial numbers in soil (Burge, 1969). Expression of the action of catechol-1,6-dioxygenase, a metacleaving enzyme possessed by *Achromobacter*, has been accomplished by using the technique of co-metabolism (Horvath and Alexander, 1970). Co-metabolism is especially important for the degradation of mixtures of polycyclic aromatic hydrocarbons (Chauhan et al., 2008). Strains that exhibit the phenomenon of co-metabolism in a well organized manner include *Nocardia*, *Pseudomonas*, *Xanthomonas*, *Bacillus*, *Brevibacterium*, *Flavobacterium*, *Aspergillus*, *Azotobacter*, *Trichoderma*, *Vibrio*, *Achromobacter*, *Arthrobacter*, *Hydrogenomonas*, *Microbacterium*, *Micrococcus* and S*treptomyces* (Beam and Perry, 1973).

CATABOLIC GENE ORGANIZATION

Degradation of xenobiotic compounds is frequently interfered by a network of enzymes, present in the bacterial system. This brings about constraints in the completion of the process. The manipulation of the catabolic genes from degradative enzymes could solve the problem and boost up the process. For instance, degradation of xenobiotic compounds such as atrazine and nitrotoluene is occasionally mediated through a permutation of bacterial strains, which carry assorted genes responsible for various fractions of the pathway (De Souza et al., 1998). The degradation mainly depends upon the adapting response of the microbial communities which include both selective enrichment (resulting in amplification of genes) and genetic changes (mainly includes gene transfer or mutation). With the mobilization of silent sequences into the functional catabolic routes and advancement of substrate range by gradual or spontaneous mutations, the recalcitrance of several toxic synthetic pollutants would certainly decrease.

Recently, approaches have been made to assemble data with reference to adaptation in bacterial populations to specific xenobiotic compounds by gene transfer and to characterize and compare the genes involved in degradation of identical or similar xenobiotic compounds in nearly diverse or more isolated bacterial genera from



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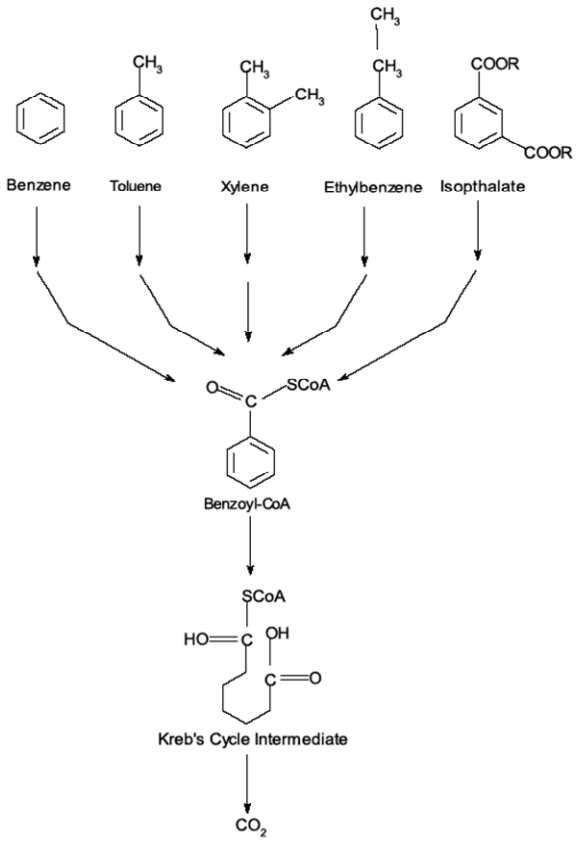

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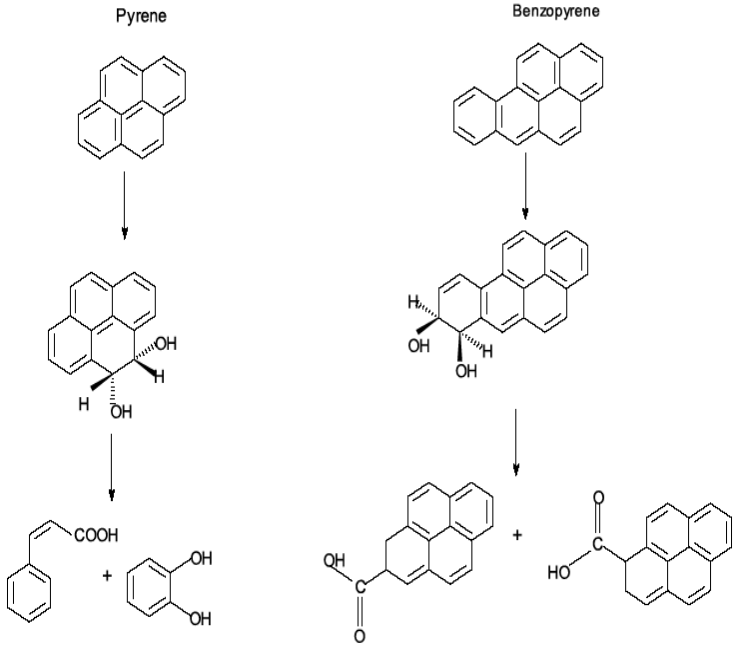

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Figure 2. Pathway of degradation of different xenobiotic compounds. a. Aliphatic compound; b. Aromatic monocyclic compound; c. Aromatic polycyclic compound.

Table 2. List of catabolic genes for biotransforming xenobiotic compounds.

Compound	Location	Genes	Source organism	References
HCH	Chromosome	*lin*A/*lin*A2/*lin*X1 *lin*B/ *lin*C/*lin*X2/*lin*X3	*Sphingobium indicum* B90A	Malhotra et al., 2007
Pthalate	Plasmid	*ptr*D/ *ptr*A/*ptr*B/*ptr*C/*pht*Aa/ *pht*Ab/*pht*Ac/*Pht*ad	*Arthrobacter keyseri* 12B	Vamsee and Phale, 2008
Napthalene	Chromosome	*pah*A/ *pah*Ab/ *pah*B/ *pah*C/ *pah*D/ *pah*E/ *pah*F	*Pseudomonas putida*	Habe and Omori, 2003; Chauhan et al., 2008
Phenanthrene	Plasmid	*phn*Aa/ *phn*Ac/ *phn*Ad/ *phn*B/ *phn*C/ *phn*D/ *phn*E/ *phn*F/ *phn*G *phn*H/ *phn*I	*Alcaligenes faecalis* AFK2	Habe and Omori, 2003; Chauhan etal, 2008
Pyrene	Chromosome	*pbh*D	*Sphingomonas paucimobilis* Var.EPA505	Kanaly and Harayama, 2000
PCB	Plasmid	*bph*A/ *bph*B/ *bph*C/ *bph*D	*Alcaligenes sp.*	Shields et al., 1985
2,4-D	Plasmid and chromosomal	*tfd*A/ *tfd*B/ *tfd*C/ *tfd*D/ *tfd*E/ *tfd*F/ *tfd*R	*Alcaligenes eutrophus* JMP134	Don and Pemberton, 1981
Chlorobenzene	Transposon	*tcb*AB	*Pseudomonas* P51	Werlen et al., 1996
Paratoluenesulfonicacid	Plasmid	*tsa*	*Comamonas testosteroni*T-2	Tralau et al., 2001
1,2,4,5-tetrachlorobenzene	Plasmid	*tec*AB	*Burkholderia* PS12	Beil et al, 1999
Phenanthrene	Chromosome	*phd*	*Nocardioides* KP7	Habe and Omori, 2003

diverse topologies. Some observations have been made:

(i) Evolutionarily related catabolic genes and their clusters have been derived from very distant locations in bacterial genera;
(ii) The phylogeny of the catabolic genes is not compatible with that of the 16S rRNA genes of the related hosts;
(iii) Genes for the degradation of synthetic pollutants are often associated with plasmids and transposons and
(iv) Evolutionary related catabolic genes and entire gene modules are involved in the degradation of structurally similar but different xenobiotic compounds (Top and Springael, 2003).

It, therefore, appears that such genes are mostly clustered and located either on chromosomes acting as insertion elements or on plasmids as mobile genetic elements, and they also facilitate horizontal gene transfer as listed in Table 2. In gram positive bacteria especially mycobacteria, genetical and biochemical data of high molecular weight, polycyclic aromatic hydrocarbon degradation is relatively lower since it possesses extremely resistant cell wall, significantly low growth rate and triggers the activity of cell clumping. In gram positive bacteria, *nid*, *pdo* genes encode high molecular weight polycyclic aromatic hydrocarbons (HMWPAH) dioxygenases, whereas in gram negative bacteria *nah*, *pah* and *phn* genes encode low molecular weight polycyclic aromatic hydrocarbons (LMWPAH) dioxygenases (Liang et al., 2006).

Apart from that, gram positive thermophilic, bacterium *Bacillus* sp. JF8N has the ability to degrade polychlorinated biphenyl compound. While studying spontaneous mutation in strain JF8N, it was found that mutant lost its ability to utilize biphenyl, but retained the ability to utilize naphthalene. This is because JF8N lost 40 Kb plasmid coding *bph* involved in degradation of PCB, and the other gene *nah* is coded by chromosomal DNA (Kimbara, 2005). Regarding the plasmids, *Moraxella*B has two: pUO1 (43Mda) and pUO11 (40MDa), responsible for the degradation of haloacetate compounds (Widada et al., 2002). The *tfd* genes, organized as *tfd*CDEF on the plasmids, pJP4 and pRC10, have been mapped, cloned and sequenced and they help in characterization of the pathway for 2,4-D degradation (Chaudhry and Chapalamadugu, 1991). The bacterial strain, *Delftia acidovorans*P4a, efficiently mineralizes 2,4-dichlorophenoxyacetic acid (2,4-D) under alkaline conditions. The screening of genomic DNA library revealed the presence of two 2,4-D gene clusters, *tfdCDEF* and $tfdC_{II}$ E_{II} BKA. It is also found that *tfdR* genes are located in the vicinity of each *tfd* gene cluster. The outcome showed that the recognized genes of the complete 2,4-D degradation pathway are organized in a single genomic unit and the entire region is flanked by insertion elements of the IS*1071* and IS*1380* families, in a chromosomally located catabolic transposon of 30 kb (Hoffman et al., 2003).

Mobile genetic elements support horizontal gene transfer more accurately via conjugation or transformation that can impart novel phenotypes or may modify existing genes through mutational processes. Typical catabolic plasmids are TOL, OCT, CAM, NAH etc. (Mishra et al., 2001). Studies have confirmed that horizontal transfer of catabolic genes, mostly by means of plasmid-mediated conjugation, happens in soil microcosms, bioreactors and so on after inoculation of a donor strain containing natural catabolic genes (Fulthorpe and Wyndham, 1992). The catabolism of benzoate and phthalate suggests high degree of redundancy, as *Rhodocccus*RHA1 has three linear plasmids (1,1,0.45 and 0.33 Mb). The smallest one has 300 putative genes and performs one fourth of the catabolic reactions, encoding 2,3-dihydroxybiphenyl 1,2-dioxygenase. This suggests that it has potential to degrade various PCB congeners (Larkin et al., 2005). Toluene degrading plasmid (TOL) is also one of the best studied catabolic plasmids, as it bears degradative genes for xylene, toluene, benzoate, salicylate, catechol, phenol and several other complex compounds (Lloyd et al., 1999).

Generally, the mechanism of gene action for degradation varies from organism to organism, depending on the entire organization as seen below.

(i) In the first category, genes are organized on single operon system e.g. phenol (Khan et al., 2001). Here the genes may be characterized by the plasmid genome or may be present separately on the chromosome.
(ii) In the second group, genes are organized on two operon systems e.g. polychlorinated biphenyls etc. Here the genes are characterized by plasmid genome (Shimizu et al., 2001).
(iii) In the third group, genes are organized on more than two operon systems e.g. 2,4-dichlorophenoxyaceticacid etc. Here, the genes are characterized by plasmid genome and the capability or incapability of bacteria to do the entire degradation depends on the existence of the complementary enzymes encoded by genes of the chromosome (Don and Pemberton, 1981).
(iv) In another group, the genes are organized on transposons, e.g. 2,4,5-trichlorophenoxyacetate (2,4,5-T is a herbicide). *Pseudomonas* AC1100 contains two insertion elements, RS110 selected as IS931 and IS932; they play a significant role in degradation of 2,4,5-T (Chaudhry and Chapalamadugu, 1991).

The expression of catabolic genes is in total when enzymes (peripheral and ring cleavages), substrate, metabolites and structure of respective genes all are in a synchronized manner (Whyte et al., 2002).

MOLECULAR APPROACHES

Most of the xenobiotic degrading bacteria harbor plasmids which code for catabolic genes. To characterize the appropriate genes and to enhance the process of degradation through improved constructed strains, a proper management is required. And for the same, degradation technology is spanning the spectrum from environmental monitoring ultimately to biodegradation as well as bioremediation (Eyers et al., 2004). Currently, molecular approaches are being used to characterize the nucleic acids of various bacteria from environmental samples (Hurt et al., 2001). Comparing with standard microbiological methods, the molecular techniques provide us with a more comprehensive interpretation of the *in situ* microbial community and its response to both engineered bioremediation and natural attenuation processes (Brockman, 1995).

PCR amplification, subsequent analysis of bacterial rRNA genes by sequencing, preparing metagenomic libraries, RFLP, dot-blot, southern blot, denaturing gradient gel electrophoresis (DGGE), microarrays are several techniques which are applied for degradation. The gene encoding TCE-RDase required for PCE biotransformation, *tceA* has been cloned and sequenced by an inverse PCR approach. Sequence comparisons of *tce*A to proteins in the public databases revealed weak sequence similarity confined to the C-terminal region, which contains the eight-iron ferredoxin cluster binding motif (Magnuson et al., 2000). Direct DNA hybridization techniques have been made to monitor TOL and naphthalene degrading plasmid (NAH) (Sayler and Layton, 1990). Polychlorinated biphenyl (PCB) catabolic genes have been used to measure the level of PCB-degrading organisms in soil microbial communities with the help of dot-blot technique (Walia et al., 1990). To quantify the degradation of 2,4-dichlorophenoxyacetic acid (2, 4-D), *tfd*A and *tfd*B gene probes have been used and identified with the help of southern hybridization technique (Holben et al., 1992).

Another powerful molecular technique known as metagenomic libraries has been flourished for the identification of the desired catabolic genes. Basically, metagenomic is a culture dependent genomic analysis; it is either function driven approach or sequence driven approach, of total microbial communities, which provides access to retrieve unknown sequences (Schloss and Handelsman, 2003). Though the technique is applicable, yet contains certain drawbacks. One of the major drawbacks is less recovery of desired clones that however can be overcome. The metagenomic libraries are particularly promising for locating denitrifying genes (Eyers et al., 2004). The sequence-driven approach which is primarily based on conserved regions in bacterial genes has also been studied. It is reported that certain hybridization probes (screen out clone libraries for specific DNA sequences) may identify the required genes for degradation. For example, in the denitration of 2,4,6-trinitrophenol, the *ndp*G and *ndp*I genes were identified in *Rhodococcus erythropolis* HL-PMI (Heiss et al., 2003).

In order to search out diverse degrading genes in

relation to bacterial ecology, fingerprinting techniques are also used which are tagged to a PCR reaction to amplify selected sequences. For example, the amplified segment of *nah*Ac genes from a miscellaneous bacterial population may be of related size when amplified with a particular set of *nah*Ac specific degenerate primers; nevertheless, they contain minute differentiation within the PCR-amplified products (Schneegurt and Kulpa, 1998). To investigate the PCB degradation, polymorphism and PCR amplification of *bph*C gene has been done, but no significant restriction polymorphism has been observed. Through DGGE and terminal restriction fragment length polymorphism (T-RFLP), a comparative analysis of PCB-dechlorinating communities in enrichment cultures have been reported (Watts et al., 2001). To evaluate the restriction fragments of PCR-amplified products, matrix-assisted laser desorption ionization time-of-flight mass spectrophotometry (MALDI-TOF-MS) has been reported (Taranenko et al., 2002). Probes with DNA microarrays have been designed to identify key genes involved in the degradation of 2,4-dichlorophenoxyacetic acid in the presence of 2,4-D with *Ralstonia eutropha*, a 2,4-D degrading bacteria (Dennis et al., 2003). Replicative limiting dilution-PCR (RLD-PCR), an alternate quantitative PCR for environmental application, is based on RLD analysis and the pragmatic trade offs between analytical sensitivity and practical utility (Chandler, 1998). This method has been used to detect and quantify specific biodegradative genes in aromatic-compound-contaminated soil. The catabolic genes *cdo*, *nah*Ac and *alk*B were used as target genes (Chandler, 1998). The biotransformation of pyrene by *Mycobacterium* KMS is extensively studied and confirmed with the aid of proteomics by identifying almost all the enzymes required during the initial steps of the degradation of this pericondensed PAH compound (Liang et al., 2006).

FUTURE PERSPECTIVE

In the past few years, there has been a great deal of progress in the study of the biodegradation of xenobiotic compounds. Several new microbes with bioremediation potential have been isolated and many new degradation pathways have been elucidated. Nevertheless, this knowledge is far from complete. The biotransformation of organo sulphide compounds is still to be explored. The efficiency of xenobiotic degradation can be significantly improved by addressing key issues as tolerance to various xenobiotics, constitutive expression of the catabolic genes and the substrate-specificity, kinetics and the stability of the encoded enzyme. However, the utility of constructed organisms in dealing with problems related to environmental pollution in nature is yet to be tested.

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