Biotechnology and Molecular Biology Review Vol. 2 (1), pp. 001-013, February 2007
Available online at http://www.academicjournals.org/BMBR
ISSN 1538-2273 © 2007 Academic Journals

Standard Review

Molecular strategies of microbial adaptation to xenobiotics in natural environment

Olusola Abayomi Ojo

Department of Microbiology, Lagos State University, Badagry Expressway, P.O. BOX 12142 Ikeja, Lagos, Nigeria. E-mail: solayom@yahooo.com. Mobile: 234-8055055478.

Accepted 28 November, 2006

The unprecedented population increase and industrial development during the twentieth century has increased conventional solid and liquid waste pollutants to critical levels as well as produced a range of previously unknown strange synthetic chemicals for which society was unprepared. Increasing pollution of the environment by xenobiotic compounds has provoked the need for understanding the impact of toxic compounds on microbial populations, the catabolic degradation pathway of xenobiotics and upgrade in bioremediation processes. Adaptation of native microbial community to xenobiotic substrates is thus crucial for any mineralization to occur in polluted environment. Enzymes which catalyze the biodegradation of xenobiotics are often produced by induction process and this subsequently determine the acclimation time to xenobiotic substrates. Microbial degraders are adapted to xenobiotic substrates via various genetic mechanisms that subsequently determine the evolution of functional degradative pathways. The ultimate goal of these genetic mechanisms is to creating novel genetic combinations in microorganisms that facilitates mineralization of xenobiotics. Moreover, recent development of high-throughput molecular techniques such as polymerase chain reaction (PCR), microarrays and metagenomic libraries have helped to uncover issues of genetic diversity among environmentally relevant microorganisms as well as identification of new functional genes which would enhance pollution abatement management in the twenty-first century.

Key words: Biodegradation, bioremediation, DNA, metagenomics, microarrays and xenobiotics.

TABLE OF CONTENTS

1. Introduction
2. Factors affecting xenobiotic degradation
3. Genes and degradation of aromatics
4. Strategies of adaptation to xenobiotics
4.1. Recombination and transposition
4.2. Gene duplication
4.3. Mutational drift
4.4. Gene transfer
5. New molecular techniques for detecting xenobiotic-degrader
5.1. Metagenomic libraries
5.1.1. Limitations of Metagenomics libraries
5.2. Microarrays
5.2.1. Limitations of Microarray technology
5.3. Complementary roles of function-driven and sequence-driven approaches
6. Conclusion
7. References

INTRODUCTION

Xenobiotics are chemically synthesized organic compounds most of which do not occur in nature (Schlegel, 1995). Xenobiotics are defined as compounds that are foreign to a living organism. Where these compounds are

not easily recognized by existing degra-dative enzymes, they accumulate in soil and water (Esteve-Nunez et al., 2001). Xenobiotics include fungi-cides, pesticides, herbicides, insecticides, nematicides, and so on. Most of which are substituted hydrocarbons, phenyl carbonates, and similar compounds. Some of these substances of which great quantities are applied to crops and soil are very recalcitrant and are degraded only very slowly or not at all. Therefore, the discovery of a new catabolic pathway to complete mineralization of the pollu-tant would be much desirable. Synthetic fibres like polyethylene and polypropylene, though harmless, are practically non-degradable. Whilst the plastizers and softeners contained in textiles are gradually oxidized, the polymer skeleton remains intact (Schlegel, 1995). Among the xenobiotics, polyaromatic, chlorinated and nitro-aromatic compounds were reported to be toxic, mutagenic and carcinogenic for living organisms. However, microbial diversity and versatility for adaptation to xenobiotics makes them the best candidates among all living organisms to convey xenobiotic compounds into natural biogeochemical cycles. Although, more microorganisms are being described as able to degrade these anthropogenic molecules, some xenobiotics have been shown to be unusually recalcitrant (Esteve-Nunez et al., 2001).

The discovery of new catabolic pathways leading to mineralization of this pollutant would be more valuable and afford a better knowledge of the diversity of catabolic pathways for the degradation of xenobiotics as well as bring valuable information for bioremediation processes (Black, 1999). The large majority of the earth's microorganisms remain uncharacterized because of the inability to isolate and cultivate them on appropriate media. Although, cultivation techniques are improving, the scientific knowledge of their growth conditions in nature (that is, chemistry of the original environment, life in complex communities, obligate interactions with other organisms, etc.) remains insufficient to cultivate most of these microorganisms (Leadbetter, 2003). This is particularly true in complex biological systems like soils, where, despite a huge bacteria diversity (up to 10^{10} bacteria and probably thousands of different species per gram of soil) (Rosello-Mora and Amann, 2001).Whereas less than 1% of bacteria has been cultured so far (Torsvik and Qvreas, 2002).

Several different molecular methods independent of cultivation have been developed to explore the diversity of microorganisms, cultivable or not, in natural environment.

Most of these methods are based on PCR amplification and subsequent analysis of bacterial rRNA genes by sequencing and fingerprint methods (clone libraries, restricttion fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), denaturing gradient gel electrophoresis (DGGE, etc.). The discovery of many new bacterial lineages and the reassignment to the most ecologically significant group when using these methods have led to a dramatic change in our perception of microbial diversity and phylogenetic tree of life (Ward et al., 1990; Amann et al., 1995).

Venter et al. (2004) using a cultivation-independent molecular approach, found thousands of new bacterial species and more than one million new protein-coding genes in 2001 of Sargasso seawater. Thus, corroborating the fact that there are millions of genes, uncharacterized microorganisms and other protein-coding genes yet to be discovered, thus, presenting a tremendous potential for the discovery of new antibiotics, secondary metabolites or xenobiotic degradation pathways. El–Fantroussi (2000) also reported similar discovery while studying the biodegradation of Linuron, a herbicide where the majority of the microbial species involved in the biodegradation were difficult to culture, but were detectable by denaturing gradient gel electrophoresis (DGGE).

The development of species-specific oligonucleotide primers and polymerase chain reaction (PCR) can now be used as a confirmatory assay for microbial isolates, since it's highly sensitive if conducted with one of the real-time technologies (Schaad et al., 1999). DNA array technology, essentially a reverse dot blot technique, is an emerging methodology useful for rapid identification of DNA fragments and may be applicable for rapid identification and detection of plant pathogens (Levesque, 1997, 2001). An array of species-specific oligonucleotide probes representing the various pathogens of potato, built on a solid support such as a nylon membrane or microscope slide, could be probed readily with labelled PCR products amplified from a potato sample. This is done by using conserved primers to amplify common bacterial genome fragments from extracts of potato tubers that might contain the bacterial pathogens, the presence of DNA sequences indicative of pathogenic species would be revealed by hybridization to species-specific oligonucleotide probes within the array (Fessehaie, 2003). This has been done using conserved ribosomal primers and labelled simultaneously with digoxigenin-dUTP. Hybridization of amplicons to the array and subsequent serological detection of digoxigenin label revealed different hybridization patterns that were distinct for each species and subspecies tested (Fessehaie, 2003).

These recently developed molecular methods have the capacity to explore simultaneously the astonishing taxonomic and functional variety among microorganisms. The metagenomic libraries are constructed from the environmental genome and clones are screened either for a desired trait ("function –driven" approach) or for a specific sequence ("sequence – driven'' approach) (Schloss and Handelsman, 2003). These molecular techniques would enhance several aspects of environmental biotechnology, spanning the spectrum from environmental monitoring (Guschin et al., 1997) to bioremediation and biodegradation (Dennis et al., 2003).

The objective of this review is to emphasize the potency of application of new molecular biology techniques

such as polymerase chain reaction (PCR), micro-arrays and metagenomic libraries for assessment of the genetic diversity among environmentally relevant micro-organisms and identification of new functional gene involved in the catabolism of xenobiotics.

FACTORS AFFECTING XENOBIOTIC DEGRADATION

Microorganisms are ubiquitous; hence they have the capacity to adapt to xenobiotic compounds as novel growth and energy substrates. The pollution of the environment with synthetic organic compounds has become an issue of public health concern. Many harmful synthetic organic compounds, which are slowly degradable, have been identified; this includes halogennated aromatics (such as benzenes, biphenyls and anilines), halogenated aliphatics and several pesticides (Spain and Van Veld, 1983). Several factors may be responsible for the slow biodegradation of these compounds in the natural environment; this may include unfavourable physio-chemical conditions (such as temperature, pH, redox potential, salinity and oxygen concentration), presence of alternative nutrients, the accessibility of the sub-strates, or predation (Goldstein et al., 1985). Slow biode-gradation of xenobiotics may also occur due to absence of genetic information coding for appropriate catabolic enzymes or proteins in native microbiota.

However, microbial communities exposed to strange synthetic organic compounds over a long period often metabolize them completely (Rieger et al., 2002). Although, adaptive acquisition of degradative abilities by bacteria for some organic compounds or the resistance to heavy metals induced after long acclimation period in laboratory-simulated ecosystems has been observed (Aelion et al., 1987). Acclimation to xenobiotics may be due to;

(i) Induction of specific enzymes among microbial community which enhanced the degradative capacity of the entire community (Spain and Van Veld, 1983),
(ii) Development of a specific sub-population of microbial community with capacity for co-metabolic process with the main microbial population,
(iii) Adaptation can also be due to the selection of mutants which acquired altered enzymatic specificities or novel metabolic activities and which were not present at the onset of the exposure of the community to the introduced compounds (Barkay and Pritchard, 1988; Timmis and Pieper, 1999).

Such a selective process (that is, induction, growth and mutation) may be responsible for the adaptation observed in mineralization of recalcitrant xenobiotics. (Haigler et al., 1988; Timmis and Pieper, 1999).

Van der Meer et al. (1992) critically analyzed some of these genetic mechanisms by which microorganisms adapt to xenobiotics that includes genetic recombination, transposition, mutational drift and gene transfer. These genetic strategies accelerate the processes of evolution of catabolic pathways in bacteria. The analysis of sequence information showed divergence of micro-organisms isolated from geographically separated areas of the world but harbouring the catabolic genes for xeno-biotics. Haigler et al. (1988), Timmis and Pieper, (1999) attributed the genetically influenced selective process as being the underlying principle behind mineralization of recalcitrant halogenated aromatics.

GENES AND DEGRADATION OF AROMATICS

Aromatic compounds carrying substituents forms a special class of xenobiotics because of their recalcitrance. Often reported are the aerobic processes of mineralization whereas anaerobic processes of biode-gradation do occur in natural environment (Reineke, 1984; Becker et al., 1999). A general comparison of the major pathways for catabolism of aromatic compounds in bacteria has revealed that the initial biotransformation steps are mediated by different enzymes that subse-quently produce limited number of central intermediates such as protocatechuates and substituted cathecols (Reineke, 1984). These dihydroxylated intermediates are channelled into either a '*meta* cleavage or o*rtho cleavage'* pathway (Haigler et al., 1988).The *ortho* cleavage pathways are involved in the degradation of catechol and protocatechuate (Doten et al., 1987). Moreover, the enzymes involved in the mineralization of chlorocathecols (that is, substituted catechols) have wider substrates specificities; hence they are rather referred to as the modified *ortho* cleavage pathway (Figure1). This parti-cular pathway has been detected in *Pseudomonas* sp. Strain B13; *Alcaligenes eutrophus* JMP134 among many others which metabolize chlorinated benzenes (Haigler et al., 1988). The Modified *ortho* cleavage pathway genes for three bacteria species were extensively studied:

(i) The *clc* ABD operon of *Pseudomonas putida* (pAC27) (Ghosal and You, 1989).
(ii) The *tfdCDEF* operon of *A. eutrophus* JMP134 (Pjp4) (Don et al., 1985).
(iii) The *tcbCDEF* operon of *Pseudomonas* sp. strain P51 (pP51) (Van der Meer et al., 1991).

The outcome of these studies and many others corroborated the fact that the genes for the modified *ortho* cleavage pathways are generally located on catabolic plasmids and their organization into operon structures was contrary to that of the chromosomally encoded *cat* and *pca* genes (Don et al., 1985).The *cat* and *pca* genes encodes for the *ortho* cleavage pathway enzymes, they are located on the chromosomes (Doten et al., 1987; Neidle and Ornston, 1986).

STRATEGIES OF ADAPTATION TO XENOBIOTICS

The spontaneous occurrence of DNA rearrangements in xenobiotic-degraders that resulted in evolution of different pathways for mineralization of synthetic compounds in natural environment is one of the principal mechanisms of adaptation to xenobiotic substrates. The evolution of catabolic pathways (that is, modified *ortho* cleavage path-way, *meta* cleavage pathway and others) in micro-organi-sms for xenobiotic substrates often involves different gene clusters encoding for the aromatic path-way enzy-mes.

RECOMBINATION AND TRANSPOSITION

Recombination is the combining of genes (DNA) from two or more different cells. This is principally based on molecular methods involving cutting of DNA fragments from different cells harbouring desired catabolic traits. These DNA fragments through hybridization in host cells that are known as recombinants, is seeded onto polluted environment where the expression of the cata-bolic trait is desired (Black, 1999). This strategy is often practiced in vitro than in vivo. El-Fantroussi (2000) in the soil enrichment method for the degradation of an herbi-cide (Linuron) engaged a modified strategy which can be extended for bioremediation process in soil polluted by this herbicide.

In another biodegradation involving the use of *Acine-tobacter* and *Pseudomonas* species, DNA rearrangement strategy was used to achieve mineralization. The orders of the genes encoding the *ortho* cleavage pathways of *Acinetobacter calcoaceticus* and *P. putida* differ from one another and from those of other organisms, suggesting that various DNA rearrangements have also occurred (Van der Meer et al., 1991). Gene rearrangements are also evident even between the different operons for the modified *ortho* pathways enzymes (Figure 1). There are as yet no clear indications of what mechanisms may direct these rearrangements. Rearrangement of DNA fra-gments is believed to be due to insertion elements which subsequently enhance gene transfer as well as activation or inactivation of silent gene (Tsuda et al., 1989).

GENE DUPLICATION

This is an important mechanism for the evolution of different strains of microorganisms of the same species. Once a gene becomes duplicated, the extra gene copy thus becomes independent of selective pressures and subsequently imbibes mutations with speed. These muta-tions could eventually lead to full inactivation, rendering this copy silent. Reactivation of the silent gene copy could then occur through the action of insertion elements. This occurred in *Flavobacterium* sp. Strain K172 that pro-duced two isozymes of 6-aminohexanoate dim-mer hydrolase, one of the enzymes involved in the degra-dation of nylon oligomers (Okada et al., 1983).

MUTATIONAL DRIFT

Mutational drift in terms of point mutation is of much rele-vance in xenobiotic degradation. It is possible that a number of stress factors such as chemical pollutants in-duce error-prone DNA replication that subsequently acce-lerates DNA evolution.

Point mutation involves base substitution, or nucleotide replacement, in which one base is substituted for another at a specific location in a gene (Black, 1999). This kind of mutation changes a single codon in mRNA, and it may or may not change the amino acid sequence in a protein. Several examples have illustrated that single-site-muta-tions can alter substrate specificities of enzymes or effector specificities. Clarke (1984) isolated mutants with altered substrate specificities of the AmiE amidase of *Pseudomonas aeruginosa*, which were provoked by single-base-pair changes. Sequential mutations in the cryptic *ebg* genes of *Escherichia coli* were shown to result in active enzymes capable of metabolizing lactose and other sugars.

Single-site-mutations are believed to arise continuously and at random as a result of errors in DNA replication or repair. Although, the important effects of single-base-pair mutation on the adaptive process have been demon-stra-ted experimentally, the accumulation of the single-base-pair changes may not be the main reason for the differ-ences in the properties of the catabolic enzymes elicited by xenobiotic-degraders. There are other factors that would precipitate changes in DNA sequences, this inclu-ded gene conversion or slipped-strand mispairing (Niedle et al., 1988).

GENE TRANSFER

Gene transfer is a process of movement of genetic infor-mation between organisms (Black, 1999). The impor-tance of gene transfer for adaptation of host cells to new compounds has been explicitly demonstrated in many studies on experimental evolution of novel meta-bolic activities. Such studies identified biochemical block-ades in natural pathways that prevented the degradation of novel substrates and these barriers scaled by trans-ferring appropriate genes (Reineke et al., 1982).

Genetic interactions in microbial communities are effe-cted by several mechanisms such as conjugation via plasmid replicons, transduction and transformation (Saye et al., 1990). The occurrence of plasmids in bacteria in the natural environment is certainly a general phenol-menon and an important pool of genetic information resi-ding on plasmid vehicles may flow among indigenous organisms. The self transmissible plasmids that carry

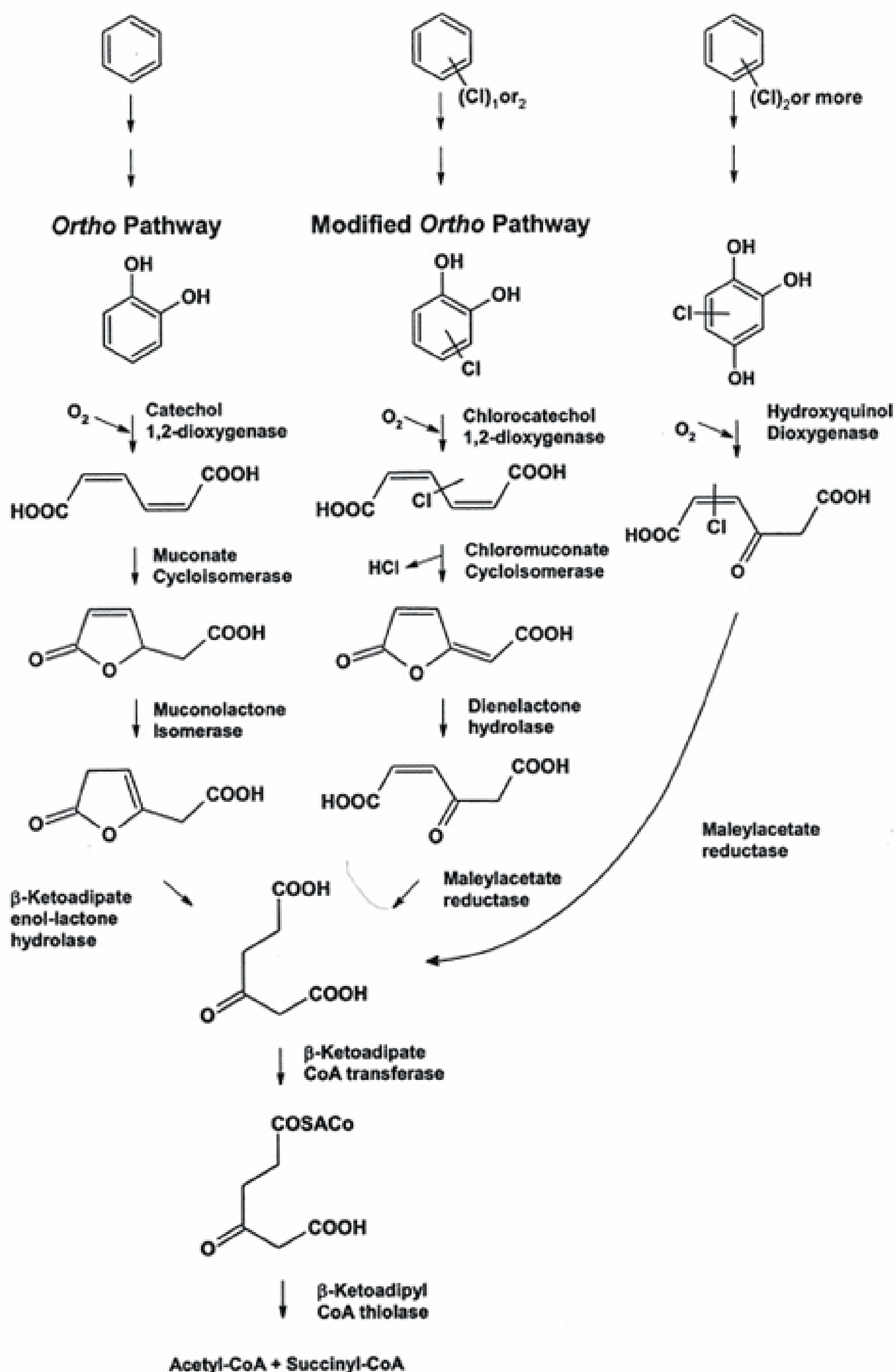

Figure 1. Modified *ortho* cleavage pathway. Adapted from Ferraroni et al. (2004).

genes for degradation of aromatic or of other organic compounds are known and their roles in spreading these genes to other organisms is predictable (Assinder and Williams, 1988).

Although, the transfer of catabolic plasmids can lead to regulatory and / or metabolic problems for the cells and therefore additional mutations in the primary transconjugants are often needed to construct strains with the desired metabolic activities (Reineke et al., 1982).

NEW MOLECULAR TECHNIQUES FOR DETECTING XENOBIOTIC- DEGRADER

There are many microorganisms in natural environment

that can degrade biphenyls, halogenated aromatics, naphthalene and xylenes. However, the assessment of the distribution of these microorganisms exhibiting specific genetic traits has been handicapped due to the fact that a large proportion are not culturable and some genes are latent.The discovery of DNA-DNA hybridization technique that is a relatively novel experimental app-roach in environmental biotechnology provided the solution to the problems of culturability and gene expression among microorganisms (Sayler and Layton, 1990; Leadbetter, 2003). Fessehaie et al. (2003) obtained oligonucleotides from bacteria pathogenic on potato which he designed and formatted into an array by pin spotting on nylon membranes. Genomic DNA from bacterial cultures was amplified by polymerase chain reaction (PCR) using conserved ribosomal primers and labeled simultaneously with digoxigenin-dUTP. Hybridization of amplicons to the array as well as detection of digoxigenin label showed different hybridization patterns that were distinct for each species and subspecies tested. DNA array technology is essentially a reverse dot blot technique useful for identification of DNA fragments and this was applied for rapid identification and detection of bacteria pathogenic on potato. An array of species-specific oligonucleotide probes representing the various pathogens of potato was constructed on a solid support (that is, nylon membranes). This was probed with labelled PCR products amplified from a potato sample, mean-while, conserved primers to amplify common bacterial genome fragments from extracts of potato tubers that had previously been infected by different bacterial pathogens was generated. The presence of DNA sequences indicative of pathogenic species would be shown by the hybridization to species-specific oligonucleotide probes within the array. This discriminatory technology identifies genomic DNA fragments of bacterial pathogens via the distinct species-specific hybridization patterns shown using the gel electrophoresis. Specific DNA sequences of native microorganisms could also be detected in environmental sources by hybridization with probes after amplification of those sequences using PCR technique (Bej et al., 1990; Weisburg et al., 1991; Eyers et al., 2004). Theoretically, the use of conserved DNA sequences in a gene family as universal primers in polymerase chain reaction amplification and consequent cloning of the amplified fragments would facilitate the detection and isolation of a wider variation of genotypes from the environment (Van der Meer et al., 1991). Weisburg et al. (1991) used this method for the characterization of variations in 16S rRNA genes from microorganisms in natural communities.

METAGENOMIC LIBRARIES

Metagenomics is the culture-independent genomic analysis of entire microbial communities (Schloss and Handelsman, 2003). In other words, metagenomics provides access to the pool of genomes of a given environment. While direct genomic cloning gives access to retrieve unknown sequences or functions in a given ecosystem that may be used for the design of primers. The PCR amplification requires prior knowledge of the sequences of genes for the design of primer. Total genomic DNA is extracted from the environment (Figure 2A) before metagenomic libraries can be constructed. The genomic DNA is enzymatically or mechanically fragmented. Fragments can be separated on the basis of their size by pulsed field gel electrophoresis (PFGE). This methodology permits fragments of an appropriate size to be isolated from the gel and inserted into host cell by cloning vectors (bacteriophage lambda, cosmid, fosmid or bacterial artificial chromosome (BAC) vectors). BAC vectors are quite efficient in maintaining stably large DNA inserts (up to 300 kb) in low copy numbers in the host cells (1-2 per cell) (Shizuya and Simon, 1992; Rondon et al., 2000). Metagenomic libraries can be screened for functional and / or genetic diversity. New catabolic genes for the degradation of xenobiotics are discovered via the “functional approach”. Clones are screened for a desired trait on appropriate media. For example, haloaromatic compounds could be used as sole electron acceptors since it has been reported that bacteria can metabolize them. (El-Fantroussi et al., 1998; Van de Pas et al., 2001). Whereas polyaromatic hydro-carbons could be utilized as sole C-source and energy-source, this often occurs after several years of adaptation that has led to a selection of a bacterial consortium cap-able of completely mineralizing such compounds. The biodegradation of linuron, a commonly used herbicide was monitored by enrichment process, using reverse transcription-PCR and denaturing gradient gel electrophoresis (DGGE), it was revealed that a mixture of bacterial species was involved in the mineralization. Although, these bacterial species appear to be difficult to culture since they were detectable by DGGE but were not cultivable on agar plates (El-Fantroussi, 2000).

Consequently, growth measurements could identify clones bearing catabolic genes. This function-driven screening remains a straightforward and successful method for the discovery of catabolic genes, as opposed to inferring the function of cloned genes by searching for homologous sequences available in database (Rondon et al., 2000). Indeed, sequences coding for important metabolic functions are frequently poorly conserved, making the comparison of clone sequences with homologous ones very difficult. This functional screening approach has been successfully used to identify novel and previously undescribed genes coding for antibiotics, lipases, enzymes for the metabolism of 4-hydroxybutyrate and genes encoding biotin synthetic pathways (Schloss and Handelsman, 2003). Most of the catabolic genes known to date were isolated from cultivable microorganisms (Table 1) (Eyers et al., 2004). However, no genes for TNT denitration have been discovered up till now. But high denitration activities were obtained under

Table 1. Properties of selected identified genes involved in degradative pathways of recalcitrant substituted aromatic compounds.

Target compound	Function	Name	Target metabolite in pathway	Mode of isolation	Size	Microorganism of origin	References
Naphthalene, Toluene, Anthracene Polycyclic aromatic hydrocarbon(PAH)	Reductase	*NL1*	PAH	Genomic library expressed in *Sphingomonas* strains	160 – 195kb	*Sphingomonas subterranean, S. aromaticivorans* F199,B0695 *S. xenophaga BN6, S.* sp. HH69	Basta et al., (2005)
2-Nitrotoluene	Reductase Ferrodoxin Iron-sulphur protein α Iron-sulphur protein β	*ntdAa* *ntdAb* *ntdAc* *ntdAd*	2-Nitrotoluene	Genomic library expressed in *E. coli* and screening for metabolic activities	4.9 kb	*Pseudomonas* sp. JS42	Parales et al., (1996)
2,4-Dinitrotoluene	Dioxygenase Monooxygenase Dioxygenase Isomerase/hydrolase Dehydrogenase	*dntA* *dntB* *dntD* *dntG* *dntE*	2,4-Dinitrotoluene 4-Methyl-5-nitrocatechol 2,4,5-Trihydroxytoluene 2,4-Dihydroxy-5-methyl-6-oxo-2,4-hexadecanoic acid Methylmalonic acid semialdehyde	Genomic library expressed in *E. coli* and screening for metabolic activities	27 kb	*Burkholderia cepacia* R34	Johnson et al., (2002)
2,4,6-Trinitrophenol	Hydride transferase Hydride transferase NADPH reductase	*npdI* *npdG* *npdC*	2,4,6-Trinitrophenol Hydride-Meisenheimer complex of 2,4,6-trinitrophenol 2,4,6-Trinitrophenol and its hydride-Meisenheimer	Genomic library and selection of clones thanks to mRNA differential display experiments	12.5kb	*Rhodococcus erythropolis* HL PM-1	Walters et al.., (2001) ; Heiss et al., (2002)
2,4,6-Trinitrotoluene	Reductase	*xenB*	2,4,6-Trinitrotoluene	Genomic library expressed in *E. coli* and screening for metabolic activities	1.05kb	*P. fluorescens* I-C	Blehert et al,., (1999)
2,4,6-Trinitrophenol	NADPH F_{420} Reductase Hydride transferase II	*npdG* *npdI*	2,4,6-Trinitrophenol Hydride-Meisenheimer complex of 2,4,6-trinitrophenol	Genomic library and selection of clones***	12.5kb	*Rhodococcus erythropolis* HL PM-1	Heiss et al., (2003)

Table 1. contd.

4-Nitrotoluene	Monooxygenase	*ntnMA*	4-Nitrotoluene	Genomic library screened with designated probes; activities confirmed by cloning and expression in *Escherichia coli.*	14.8kb	*Pseudomonas* sp. TW3	James and Williams (1998); James et al. (2000)
	Nitrobenzyl alcohol dehudrogenase	*ntnD*	4-Nitrobenzyl alcohol				
	Nitrobenzadehyde dehudrogenase	*ntnC*	4-Nitrobenzadehyde	Genomic library expressed in *P. putida*PaW340 and screening for metabolic activities			
	Nitrobenzoate reductase	*pnbA*	4-Nitrobenzoate		6 kb	*Pseudomonas* sp. TW3	Hughes and Williams (2001)
	Hydroxylaminobenzoate lyase	*pnbB*	4-Hydroxylaminobenzoate				

defined conditions with a TNT-contaminated soil sample (Eyers et al., 2004). Further more, the presence of a specific bacterial consortium in this polluted soil was demonstrated by DGGE (Eyers et al., 2004).Therefore, metagenomic libraries are particularly promising for identifying denitration genes, compared with methods based on isolation of pure cultures.

Comparatively, the sequence–driven approach of the metagenomic libraries is based on conserved regions in microbial genes. Clone libraries are screened for specific DNA sequences by means of hybridization probes and PCR primers, whose design is based on the information available in databases. Such hybridization probes may use, in the case of denitration of 2,4,6-trinitrophenol or other electron-deficient aromatics, the *npdG* and *npdI* sequences of *Rhodococcus erythropolis* HL-PM1(Table 1), as homologous sequences that have been identified in other nitroaromatic compounds-degrading *Rhodococcus* strains (Heiss et al., 2003). These sequences are clustered separately from the related enzymes. Hence, it was suggested that they may be suitable as genes probes for finding bacteria in the environment with the ability to "hydrogenate" electron-deficient aromatic ring systems (Heiss et al., 2003).

LIMITATIONS OF METAGENOMIC LIBRARIES

Although, metagenomic libraries constitute at present the most powerful tool to assess the functional diversity of natural microbial communities, they do not cover genomes of low abundance in complex environment like soils, which can be responsible for an important degradation or related process. Hence, the frequency of clones of a desired nature in a library can be very low. This implies screening thousands of clones, which can be laborious and time-consuming. High- throughput equipment is now available to facilitate colony picking, inoculation in microtitre plates and screening numerous clones at the same time. However, an enrichment strategy is required before the construction of the library to select a specific feature and in that way ensure better cover for a subset of the community (Entcheva et al., 2001). Communities enriched naturally by long-term exposure to high concentrations of xenobiotics may host the genes of interest at a high frequency. In this context, metagenomics might shorten the time required to understand the genetics of degradation.

Moreover, the catabolism of specific xenobiotics may be achieved by two or more bacteria via co-metabolic process (Abraham et al., 2002). In this case, it is not possible to isolate a contiguous piece of DNA containing all the genes involved in the catabolic pathway. Therefore, Schloss and Handelsman (2003) suggested studying multiple clones simultaneously on liquid media in which substrates and products can diffuse freely among members of the mixture. Finally, it is important for functional screening that catabolic genes are effectively expressed. Hence, another challenge lies in choosing an appropriate host of expression. Moreover, the host has to be both relatively insensitive to the toxic xenobiotic and unable to catabolize it in the absence of the vector.

MICROARRAYS

DNA chip is an emerging technique making it possible to analyze hundreds and even thousand of genes at the same time, thus getting away from the ''one gene at a time'' analysis. This allows extremely small amount of biomolecules (RNA, cDNA, etc) to be printed at high density on a substrate. Comparatively, microarrays present the advantage of miniaturization (thousands of probes can be spotted on a chip), high sensitivity and rapid detection which is not obtainable with the traditional nucleic acid membrane hybridization. Zhou and Thompson (2002) has reported that microarray –based genomic technologies would revolutionize the analysis of microbial community structure, function and dynamics. DNA microarrays are coated glass microscope slides onto which thousands of target DNA sample are spotted in a precise pattern. There are two principal microarrays types based on the nature of the target (DNA, e.g., cDNA, PCR products, oligonucleotides or plasmids, or RNA) and on the method of spotting (mechanical microspotting or photolithography). In the first method, purified DNA is spotted onto the membrane or coated glass slide. Although, DNA will stick to glass, aminosilane-coated and poly-L-lysine (PLL)-coated slides are predominantly used. Silanized slides ($RSiX_3$, where X is typically alkoxy) attach the DNA by forming covalent bonds between primary amines on the surface and the phosphate backbone (Celis et al., 2000; Ye et al., 2001). The photolithography method uses an ultraviolet light source that passes through a mask where a photochemical reaction takes place on a siliconized glass surface (Affymetrix). This is by far the most efficient method of generating high-density oligonucleotide chips, but has practical limitations in terms of fragment length and affordability (Kumar et al., 2000). After printing the microarrays, therefore, extraction of DNA or messenger RNA from pure cultures or the environment (Figure 2B), labelling it with specific fluorescent molecules and hybridizing it to target DNA spotted on the glass slide. The resulting image of fluorescent spots is visualized by confocal laser scanning and it's digitized for quantitative analysis.

Fessehaie et al. (2003) designed and formatted into an array by pin spotting on nylon membranes, genomic DNA from bacterial cultures which was amplified by PCR using conserved ribosomal primers and labelled simultaneously with digoxigenin –dUTP. Hybridization of amplicons to the array and subsequent serological detection distinctly confirmed the identity of each species of potato pathogens within the mixed cultures and inoculated potato tissues.

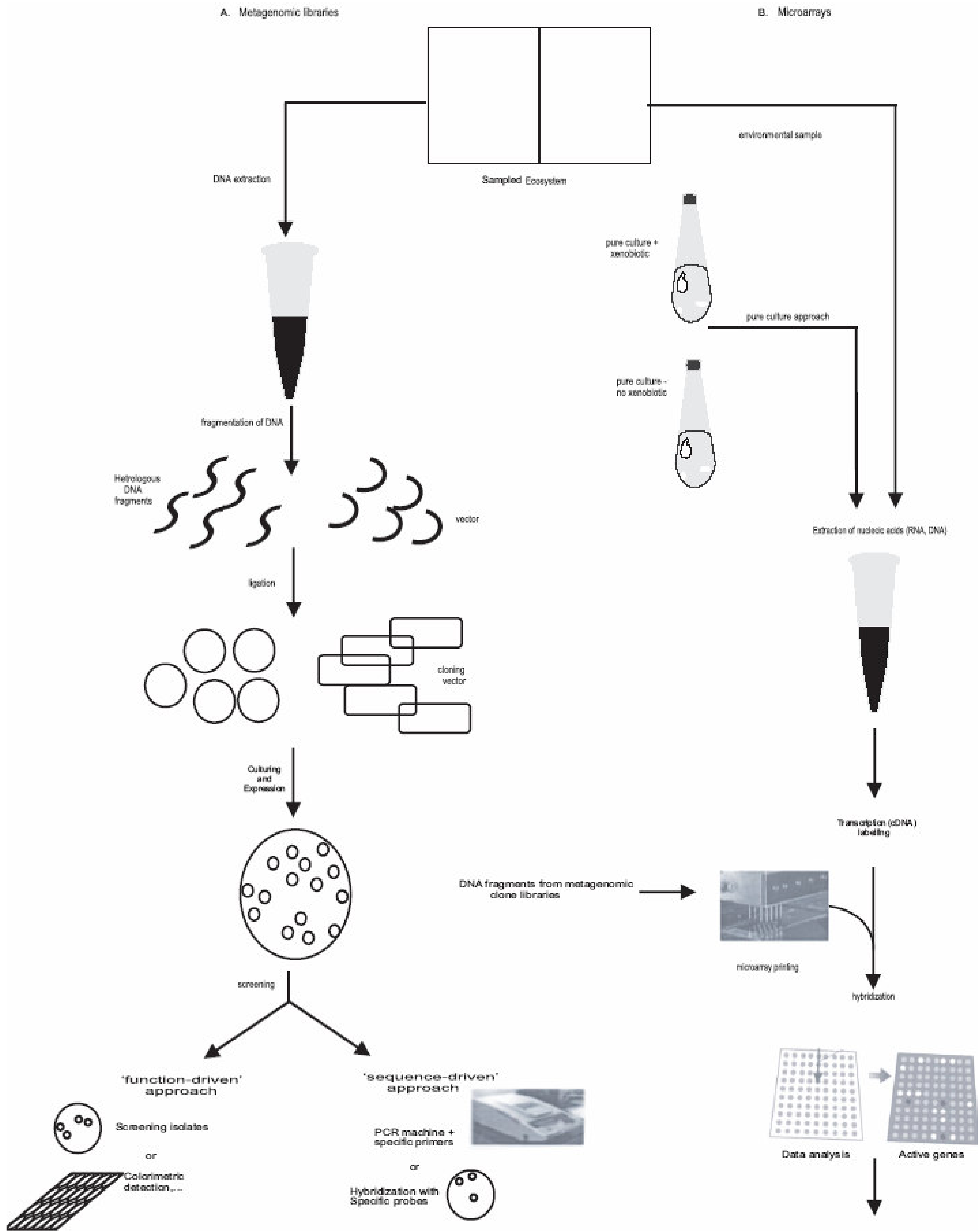

Figure 2. (A). Construction and analysis of metagenomic libraries from sampled ecosystems. (B). Construction of microarrays and hybridization with samples from the environment or pure cultures.

DNA microarrays is applied in research for gene expression profiling, that is, identification of changes in mRNA expression of strains exposed to a particular substrate, for example, a specific xenobiotic. Schut et al. (2001) constructed a DNA microarray with probes targeting 271 open reading frames (ORFs) from the genome sequence of the hyperthermophile *Pyrococcus furiosus*. When this strain was grown with elemental sulphur (S), two previously uncharacterized operons were identified and their products were proposed to be part of a novel S-reducing, membrane-associated, iron-sulphur cluster-containing complex. DNA microarray was also used to assess the mRNA expression levels in *Bacillus subtilis* grown under anaerobic condition (Ye et al., 2000). Transcriptional activities of more than 100 genes affected by the oxygen–limiting conditions were identified (Ye et al., 2000). Microarray has also been used for physiological studies of environment samples. Wu et al. (2001) detected genes involved in nitrogen –cycling (nirS) using 1ng of labelled genomic DNA of a *Pseudomonas stutzeri* strain and 25 ng of bulk community DNA extracted from soil samples. Catabolic genes involved in degradation of xenobiotics were assessed with microarrays by Dennis et al. (2003).

In microarray technology, sequence information is needed to design probes. However, this approach cannot be applicable for discovering new catabolic genes for which no sequences are available in databases. Moreover, knowledge of the entire sequence is not necessary for the construction of microarrays; and PCR products of a random genomic library constructed from a microorganism of interest may be used. It is expected that to a toxic substrate, differential gene expression would result at the transcript level. This is reflected by differential hybridization patterns in the presence or absence of the toxic pollutant. Afterward, clones of the library associated with differentially hybridized probes can be picked up for sequencing. This methodology applied in identifying genes involved in N_2 –fixation in *Leptospirillum ferrooxidans* by Parro and Moreno-Paz (2003). Sebat et al. (2003) used metagenomes as microarrays by deriving cosmid library from a microcosm of groundwater and used this library as probes for microarrays. Afterwards, they hybridized the microarrays with cDNA of individual strains isolated from the microcosm and cDNA of the microcosm itself. Comparisons of the hybridization profiles of the microcosm with isolated strains, clones were identified in the library corresponding to uncultured members of the microcosm. Sequencing of these clones revealed ORFs assigned to functions that have potential ecological importance, including hydrogen oxidation,NO_3-reduction and transposition (Sebat et al., 2003).

Therefore a random genomic library approach associated with microarrays offers a high potential for the discovery of novel genes and operons. This is desirable since it offers additional information apart from the biology of the microorganisms but precise knowledge of biodegradation processes of xenobiotics for application in pollution control and prevention under field conditions.

LIMITATIONS OF MICROARRAY TECHNOLOGY

Presence of humic matter, organic contaminants and metals in environmental samples that may interfere with RNA and DNA hybridization (Zhou and Thompson, 2002). Possibility of extraction of undegraded mRNA is additional problem (Burgmann et al., 2003). In cases of sequences of poor abundance, microarrays are not superior in sensitivity to PCR that is 100 to 10,000 fold more than that of microarrays (Zhou and Thompson, 2002). The success of the application of microarray technology in a study lies in the possibility of determining, in complex environments, the relative abundance of a microorganism bearing a specific functional gene. Therefore, it is important to be able to differentiate between differences in hybridization signals due to population abundance from those due to sequence divergence (Wu et al., 2001).

COMPLEMENTARY ROLES OF FUNCTION-DRIVEN AND SEQUENCE-DRIVEN APPROACHES

In the case of metagenomic libraries, sequencing clones with interesting functional properties may reveal a sequence that can be used to confirm the phylogenetic affiliation of the organism from which the DNA was isolated. Where the sequencing clones harbours an rRNA sequence in a big fragment that can lead to functional information about the microorganism from which the fragment originated (Beja et al., 2000; Liles et al., 2003; Quaiser et al., 2003). Environmental systems often contain a high diversity of bacteria, the use of labelled xenobiotics can provide information about active bacteria within a complex environmental system such bacteria incorporates radio labelled atoms into their DNA, making it denser than non-labelled DNA. By centrifugation, separation of labelled from non-labelled DNA can be achieved (Radajewski et al., 2000) and thereby distinguish the bacteria involved in the catabolic process from those which are not. This labelled DNA can be used afterwards to construct metagenomic libraries. This forms an excellent method for finding clones carrying catabolic genes.

Conclusion

It is clear that polluted sites, particularly those that are or become sources of contamination of surface and groundwater, have to be remediated. Bioremediation can be a cost-effective and ecologically suitable alternative to physical methods.

The genetic characterization of an increasing number of aerobic pathways for degradation of (substituted) aromatic compounds in different bacteria has made it possible to compare the similarities in genetic organization and in sequence, which exist between genes and pro-

teins of these specialized catabolic routes and more common pathways. Sequence information provided the scientific evidence of the occurrence of catabolic genes coding for specialized enzymes in the degradation of xenobiotic chemicals. Moreover, molecular biology evidences corroborated the fact that a range of genetic mechanisms, such as gene transfer, mutational drift, genetic recombination and transposition can accelerate the evolution of catabolic pathways in microorganisms.

Metagenomic libraries open access to the world of uncultivated microorganisms and their undescribed catabolic genes for the degradation of xenobiotics. Microarrays are also useful for the discovering new catabolic genes as well as provide the opportunity of easily monitoring catabolic genes. In both approaches, the use of radio-labelled molecules could improve the recovery and identification of microorganisms involved in biodegradation of xenobiotics. The technical challenges associated with metagenomic libraries and microarrays not withstanding, these methods presents an exceptional opportunity for discovering the scientific basis of microbial degradation of xenobiotics. The application of both metagenomic libraries and DNA microarrays in bioremediation processes will facilitate rapid transfer of genetic information between axenic and native microbial communities, thus enhancing microbial adaptation to metabolism of xenobiotics in the environment.

In order to minimize future environmental impact by xenobiotics it may be economical to develop new synthetic compounds that fit in the naturally existing catabolic potential of the microorganisms.

REFERENCES

Abraham WR, Nogales B, Golyshin PN, Pieper DH, Timmis KN (2002). Polychlorinated biphenyl-degrading microbial communities in soil and sediments. Curr. Opin. Microbiol. 5: 246-253.

Aelion CM, Swindoll CM, Pfaender FK (1987). Adaptation to and biodegradation of xenobiotic compounds by microbial communities from pristine aquifer. Appl. Environ. Microbiol. 53: 2212-2217.

Amann RI, Ludwig W, Schlefer KH (1995). Phylogenetic identification and in situ detection of individual cells without cultivation. Microbiol. Rev. **59**: 143-169.

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

Barkay T, Pritchard H (1988). Adaptation of aquatic microbial communities to pollutant stress. Microbiol. Sci. 5:165-169.

Basta T, Buerger S, Stolz A (2005). Structural and replicative diversity of large plasmids from sphingomonads that degrade polycyclic aromatic compounds and xenobiotics. Microbiology 151: 2025-2037.

Bej AK, Steffan RJ, Di Cesare J, Haff L, Atlas RM (1990). Detection of coliform bacteria in water by polymerase chain reaction and gene probes. Appl. Environ. Microbiol. 56: 307-314.

Beja O, Aravind L, Koonin EV, Suzuki MT, Hadd A, Nguyen LP, Jovanovich SB, Gates CM, Feldmanc RA, Spudich JL, Spudich EN, Delong EF (2000). Bacterial rhodopsin: evidence for a new type of phototrophy in the sea. Science 289: 1902-1906.

Black JG (1999). Bioremediation In: *Microbiol. Principles and explorations*.Pp. 751-752.

Blehert DS, Fox BG, Chambliss GH (1999). Cloning and sequence analysis of two *Pseudomonas* flavoprotein xenobiotic reductases. J. Bacteriol. 181: 6254-6263.

Burgmann H, Widmer F, Sigler WV, Zeyer J (2003). mRNA extraction and reverse transcription-PCR protocol for detection of *nifH* gene expression by *Azotobacter vinelandii* in soil. Appl. Environ. Microbiol. 69:1928-1935

Celis JE, Kruhoffer M, Gromova I, Frederiksen C, Ostergaard M, Thykjaer T, Gromov P, Yu H, Palsdottir H, Magnusson N, Orntoft TF (2000). Gene expression profiling: monitoring transcription and translation products using DNA microarrays and proteomics. FEBS Lett. 480: 2-16.

Clarke PH (1984). The evolution of degradative pathways. In: DT Gibson (ed.), Microbial degradation of organic compounds. Marcel Dekker, Inc. New York. pp. 11-27.

Dennis P, Edwards EA, Liss SN, Fulthorpe R (2003). Monitoring gene expression in mixed microbial communities by using DNA microarrays. Appl. Environ. Microbiol. 69: 769-778.

Don RH, Weightman AJ, Knackmuss HJ, Timmis KN (1985). Transposons mutagenesis and cloning analysis of the pathways for degradation of 2, 4-dichlorophenoxyacetic acids and 3-chlorobenzoate in *Alcalgenes eutorphus* JMP134 (pJP4). J. Bacteriol. 161: 85-90.

Doten RC, Ngai KL, Mitchell DJ, Ornston LN (1987). Cloning and genetic organization of the pca gene cluster from Acinetobacter calcoaceticus. J. Bacteriol. 169: 3168-3174.

El- Fantroussi S, Naveau H, Agathos SS (1998). Anaerobic dechlorinating Bacteria. Biotechnol. Prog. 14:167-188.

El-Fantroussi S (2000). Enrichment and molecular characterization of a bacterial culture that degrades methoxy-methyl urea herbicides and their aniline derivatives. Appl. Environ. Microbiol. 66(12): 5110-5115.

Entcheva P, Liebl W, Johann A, Hartsch T, Streit WR (2001). Direct cloning from enrichment cultures, a reliable strategy for isolation of complete operons and genes from microbial consortia. Appl. Environ. Microbiol. 67: 89-99.

Esteve-Nenez A, Caballero A, Ramos JL (2001). Biological degradation of 2, 4, 6-trinitrotoluene. Microbiol. Mol. Biol. Rev. 65:335-352.

Eyers L, Stenuit B, El fantroussi S, Agathos SN (2004). Microbial characterization of TNT-contaminated soils and anaerobic TNT degradation: high and unusual denitration activity. In: W. Verstraete (ed.) *Eur. Symp. Environ. Biotechnol*. 5, Oostende Belgium, pp. 51-54.

Ferraroni M, Solyanikova IP, Kolomytseva MP, Scozzafava A, Golovleva L, Briganti F (2004). Crystal Structure of 4-Chlorocatechol 1,2-Dioxygenase from the Chlorophenol-utilizing Gram-positive *Rhodococcus opacus* 1CP. J. Biol. Chem. 279(26): 27646-27655.

Fessehaie A, De Boer SH, Levesque CA (2003). An oligonucleotide array for the identification and differentiation of bacteria pathogenic on potato. Phytopathology 93: 262-269.

Guschin DY, Mobarry BK, Proudnikov D, Stahl DA, Rittman BE, Mirzabekov AD (1997). Oligonucleotide microchips as genosensors for determinative and environmental studies in microbiology. Appl. Environ. Microbiol. 63: 2397-2402.

Ghosal D, You S (1989). Operon structure and nucleotide homology of the chlorocatecol oxidation genes of plasmids pAC27 and pJP4. Gene 83: 225-232.

Goldstein RM, Mallory LM, Alexander M (1985). Reasons for possible failure of inoculation to enhance biodegradation. Appl. Environ. Microbiol. 50: 977-983.

Haigler BE, Nishino SF, Spain JC (1988). Degradation of 1, 2-dichlorobenzene by a *Pseudomonas* sp. Strain JS6. Appl. Environ. Microbiol. 54: 294-301.

Haigler BE, Spain JC (1989). Degradation of p-chlorotoluene by a mut ant of *Pseudomonas* sp. Strain JS6. Appl. Environ. Microbiol. 55: 372-379.

Heiss G, Hofmann KW, Trachtmann N, Walters DM, Rouviere P, Knackmuss H-J (2002). npd gene functions of Rhodococcus (opacus) erythropolis HL PM-1 in the initial steps of 2,4,6-trinitrophenol degradation. Microbiology 148:799-806.

Heiss G, Trachtmann N, Abe Y, Takeo M, Knackmuss H-J (2003). Homologous npdGI genes in 2, 4-Dinitrophenol-and 4-nitrophenol-degrading *Rhodococcus* spp. Appl. Environ. Microbiol. 69(5): 2748-2754.

Hughes MA, Williams PA (2001). Cloning and characterization of *pnb* genes, encoding enzymes for 4-nitrobenzoate catabolism in *Pseudomonas putida* TW3. J. Bacteriol. 183:1225-1232.

James KD, Williams PA (1998). *ntn g*enes determining the early steps

in the divergent catabolism of 4-nitrotoluene and toluene in *Pseudomonas* sp. Strain TW3. J. Bacteriol. 180: 2043-2049.
James KD, Hughes MA, Williams PA (2000). Cloning and expression of *ntnD, encoding a novel NAD (P)$^+$-independent* 4-nitrobenzyl alcohol dehydrogenase from *Pseudomonas* sp. Strain TW3. J. Bacteriol. 182: 3136-3141.
Johnson GR, Jain RK, Spain JC (2002). Origins of the 2,4-dinitrotoulene pathway. J. Bacteriol. 184: 4219-4232.
Kumar A, Larson O, Parodi D, Liang Z (2000). Silinized nucleic acids: a general platform for DNA immobilization. Nuc. Acids Res. 28: E71.
Leadbetter JR (2003). Cultivation of recalcitrant microbes: Cells are alive, well and revealing their secrets in the 21st. century laboratory. Curr. Opin. Microbiol. 6: 274-281.
Levesque CA (1997). Molecular detection tools in integrated disease management: Overcoming current limitations. Phytoparasitica 25:3-7.
Levesque CA (2001). Molecular methods for detection of plant pathogens-what is the future? Can. J. Plant Pathol. 24: 333-336.
Liles MR, Manske BF, Bintrim SB, Handelsman J, Goodman RM (2003). A census of rRNA genes and linked genomic sequences within a soil metagenomic library. Appl. Environ. Microbiol. 69: 2684-2691.
Niedle EL, Harnett C, Bonitz S, Ornston LN (1988). DNA sequence of *Acinetobacter calcoaceticus catechol* 1,2-dioxygenase-I structural gene *cat*A: evidence for evolutionary divergence of intradiol dioxygenases by acquisition of DNA sequence repetitions. J. Bacteriol. 170: 4874-4880.
Niedle EL, Ornston LN (1986).Cloning and expression of *Acinetobacter calcoaceticus* catechol 1, 2-dioxygenase structural gene *catA* in *Escherichia coli*. J. Bacteriol. 168: 815-820.
Okada H, Negoro S, Kimura H, Nakamura S (1983). Evolutionary adaptation of plasmid-encoded enzymes for degrading nylon oligomers. Nature (London) 306: 203-206.
Parales JV, Kumar A, Parales RE, Gibson DT (1996). Cloning and sequencing of genes encoding 2-nitrotoluene dioxygenase from *Pseudomonas* sp. JS42. Gene 181: 57-61.
Parro V, Moreno-Paz M (2003). Gene function analysis in environmental isolates: the *nif* regulon of the strict iron oxidizing bacterium *Leptospirillum ferrooxidans*. Proc. Natl. Acad. Sci. USA 100: 7883-7888.
Quaiser A, Ochsenreiter T, Lanz C, Schuster SC, Treusch AH, Eck J, Schleper C (2003). Acidobacteria form a coherent but highly diverse group within the bacterial domain: evidence from environmental genomics. Mol. Microbiol. 50:563-575.
Radajewski S, Ineson P, Parekh NR, Murrell JC (2000). Stable-isotope probing as a tool in microbial ecology. Nature 403: 646-649.
Reiger PG, Meier H-M, Gerle M, Vogt U, Groth T, Knackmuss H-J (2002). Xenobiotics in the environment: present and future strategies to obviate the problem of biological persistence. J. Biotech. 94: 101-123.
Reineke W (1984). Microbial degradation of halogenated aromatic compounds, In: DT. Gibson (ed.), Microbial degradation of organic compounds. Marcel Dekker, Inc. New York. pp. 319-360.
Reineke W, Jeenes DJ, Williams PA, Knackmuss HA (1982). TOL plasmid in constructed halo benzoate-degrading *Pseudomonas* strains: prevention of *meta* pathway. J. Bacteriol. 150: 195-201.
Rondon MR, August PR, Bettermann AD, Brady SF, Grossman TH, Liles MR, Loiacono KA, Lynch BA, Macneil IA, Micor C, Tiong CL, Gilman M, Osburne MS, Clardy J, Handelsman J, Goodman RM (2000). Cloning the soil metagenome: a strategy for accessing the genetic and functional diversity of uncultured microorganisms. Appl. Environ. Microbiol. 66: 2541-2547.
Saye DJ, Ogunseitan OA, Sayler GS, Miller RV (1990).Transduction of linked chromosomal genes between *Pseudomonas aeruginosa* strains during incubation *in situ* in a fresh water habitat. Appl. Environ. Microbiol. 56: 140-145.
Sayler GS, Layton AC (1990). Environmental application of nucleic acid Hybridization. Annual Rev. Microbiol. 44: 625-648.
Schaad NW, Berthier SY, Sechler A, Knorr D (1999). Detection of *Clavibacter michiganensis* subsp. *sepedonicus* in potato tubers by BIO-PCR and an automated real-time fluorescence detection system. Plant Dis. 83:1095-1100.
Schlegel HG (1986). Xenobiotics In: *General Microbiology* 6th edition Cambridge University Press N.Y. p. 433.
Schloss PD, Handelsman J (2003). Biotechnological prospects from metagenomics. Curr. Opin. Microbiol. 14:303-310
Sebat JL, Colwell FS, Crawford RL (2003). Metagenomic profiling: microarray analysis of an environmental genomic library. Appl. Environ. Microbiol. 69: 4927-4934.
Shizuya H, Simon M (1992). Cloning and stable maintenance of 300-kilobase-pair fragments of human DNA in *Escherichia coli* using an F-factor-based vector. Proc. Natl. Acad. Sci. USA. 89: 8794-8797.
Schut G, Zhou J, Adams MW (2001). DNA microarray analysis of the hyperthermophilic archaeon *Pyrococcus furiosus*: evidence for a new type of sulfur-reducing enzyme complex. J. Bacteriol. 183: 7027-7036.
Spain JC, Van Veld PA (1983). Adaptation of natural microbial communities to degradation of xenobiotic compounds: effects of concentration, exposure, time, inoculum, and chemical structure. Appl. Environ. Microbiol. 45: 428-435.
Timmis KN, Pieper DH (1999). Bacteria designed for bioremediation. *TIBTECH* 17: 201-204.
Torsvik V, Qvreas L (2002). Microbial diversity and function in soil: from genes to ecosystem. Curr. Opin. Microbiol. 5:240-245.
Tsuda M, Minegishi KI, Lino T (1989). Toluene transposons Tn4561 and Tn4563 are class II transposons. J. Bacteriol. 171: 1386-1393.
Van der Meer JR, Eggen RIL, Zehnder AJB, De Vos WM (1991). Sequence analysis of *Pseudomonas* sp. Strain P51 *tcb* gene cluster, which encodes metabolism of chlorinated catecols: evidence for specialization of catecol 1, 2-dioxygenases for chlorinated substrates. J. Bacteriol. 173: 2425-2434.
Venter JC, Remington K, Heidelberg JF, Halpern AL, Rusch D, Eisen JA, Wu DY, Paulsen I, Nelson KE, Nelson W, Fouts DE, Levy S, Knap AH, Lomas MW, Nealson K, White O, Peterson J, Hoffman J, Parsons R, Baden-Tillson H, Pfannkoch C, Rogers YH, Smith HO (2004). Environmental genome shotgun sequencing of the Sargasso Sea. Science 304: 66-74.
Walters DM, Russ R, Knackmuss H, Rouviere PE (2001). High-density sampling of a bacterial operon using mRNA differential display. Gene 273: 305-315.
Ward DM, Weller R, Bateson MM (1990). 16s rRNA sequences reveal numerous uncultured microorganisms in a natural community. Nature 344: 63-65.
Weisburg WG, Barns SM, Pelletier DA, Lane DJ (1991). 16S ribosomal DNA amplification for phylogenetic study. J. Bacteriol. 173: 697-703.
Wu L, Thompson DK, Li G, Hurt RA, Tiedje JM, Zhou J (2001). Development and evaluation of functional gene arrays for detection of selected genes in the environment. Appl. Environ. Microbiol. 67: 5780-5790.
Ye RW, Tao W, Bedzyk L, Young T, Chen M, Li G (2000). Global gene expression profiles of *bacillus subtilis* grown under anaerobic conditions. J. Bacteriol. 183: 7027-7036.
Ye RW, Wang T, Bedzyk L, Croker KM (2001). Applications of DNA microarrays in microbial systems. J. Microbiol. Methods. 47: 257-272.
Zhou J, Thompson DK (2002). Challenges in applying microarrays to environmental studies. Curr. Opin. Biotechnol. 13: 204-207.