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Use of Molecular Techniques in Bioremediation

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Abstract

In a practical sense, biotechnology is concerned with the production of commercial products generated by biological processes. More formally, biotechnology may be defined as "the application of scientific and engineering principles to the processing of material by biological agents to provide goods and services" (Cantor, 2000). From a historical perspective, biotechnology dates back to the time when yeast was first used for beer or wine fermentation, and bacteria were used to make yogurt. In 1972, the birth of recombinant DNA technology moved biotechnology to new heights and led to the establishment of a new industry. Progress in biotechnology has been truly remarkable. Within four years of the discovery of recombinant DNA technology, genetically modified organisms (GMOs) were making human insulin, interferon, and human growth hormone. Now, recombinant DNA technology and its products – GMOs are widely used in environmental biotechnology (Glick and Pasternak, 1988; Cowan, 2000). Bioremediation is one of the most rapidly growing areas of environmental biotechnology. Use of bioremediation for environmental clean up is popular due to low costs and its public acceptability. Indeed, bioremediation stands to benefit greatly and advance even more rapidly with the adoption of molecular techniques developed originally for other areas of biotechnology. The 1990s was the decade of molecular microbial ecology (time of using molecular techniques in environmental biotechnology). Adoption of these molecular techniques made scientists realize that microbial populations in the natural environments are much more diverse than previously thought using traditional culture methods. Using molecular ecological methods, such as direct DNA isolation from environmental samples, denaturing gradient gel electrophoresis (DGGE), PCR methods, nucleic acid hybridization *etc.*, we can now study microbial consortia relevant to pollutant degradation in the environment. These techniques promise to provide a better understanding and better control of environmental biotechnology processes, thus enabling more cost effective and efficient bioremediation of our toxic waste and contaminated environments.

Introduction

Environmental biotechnology is expanding rapidly, driven by the needs of society for a cleaner environment and emerging developments in biotechnology research. There is an increasing interest in environmental biotechnology owing to

a worldwide need to feed the world's growing population and to maintain clean soil, air and water (Wackett, 2000).

Biotechnology has been used since before recoded history to make food and beverages but modern biotechnology started with the discovery of the double helix structure DNA by Watson and Crick in 1953 and the availability of restriction enzymes, DNA ligases and polymerases needed to initiate genetic engineering technology (Alberts *et al.* 1999). Hence molecular techniques and the explosive development of modern biotechnology have occurred only in the past two decades.

Industrialization, economic growth, and increased standards of living have exacted a heavy toll on our environment over the last century. A major problem that as emerged over the last two decades is the dangerous accumulation of recalcitrant compounds, such as PAH_s, PCB_s, TNT, PCE, TCE *etc.*, in soil, sediment, and surface and/or ground waters as a result of chemical spills, industrial activities or careless disposal strategies. Petroleum fuel spills which are a prime example, have resulted in accumulation of petroleum products at refineries, fuel storage areas, airports, military bases, fuel distribution lines, and gasoline service stations. Thus considerable effort is being spent on developing cheap and feasible strategies for clean-up of contaminated sites (Jansson *et al.*, 2000) and a prime candidate for many types of cleanup are bioremediation technologies.

Bioremediation refers to the use of biological systems, usually microorganisms, to clean up contaminated environments. This approach had a number of advantages over physical and chemical treatment, primarily because it is lower in cost and more environmentally friendly but in addition, organic pollutants can be completely mineralized or biodegraded to simple inorganic compounds, *e.g.* CO_2, H_2O, and Cl^- using bioremediation, whereas physical and chemical processes, *e.g.* vaporization, adsorption and extraction, destroy soil structure and disturb natural processes in environment and quite often only transfer the contaminant from one environment to another, *e.g.* soil to the atmosphere (Semple *et al.*, 2001).

Bioremediation – what is it?

Bioremediation is a promising solution for the rehabilitation of contaminated soil, and enjoys a high degree of public acceptability due to its „natura" orgins. Biological remedies by using micoorganisms for pollution reduction (the application of which is referred to as bioremediation) have received increasing attention since the 1980s. Bioremediation is defined as "a managed or spontaneous process in which biological, especially microbiological, catalysis acts on pollutant compounds, thereby remedying or eliminating environmental contamination (biorestoration is a synonym) (Madsen, 1991).Though bioremediation has received a lot of attention in recent years but it is not a new technology. Composting, sewage treatment, and certain types of fermentation have been practised by humankind since the beginning of recorded history, and all of these utilize microbial processes. Evidence of kitchen middens and compost piles dates back to 6000 B.C. The more „modern" use of bioremediation began over

100 years ago with the opening of the first biological sewage treatment plant in Sussex, UK, in 1891. Yet, the word „bioremediation" is fairly new (McCullough *et al.*, 1999). Its first appearance in peer–reviewed scientific literature was in 1987 (Hazen, 1997). Bioremediation is an alternative to traditional remediating technologies, such as landfilling or incineration. It works by either transforming or degrading contaminants to non-hazardous or less hazardous chemicals. These processes are called, respectively, biotransformation and biodegradation (McCullough *et al.*, 1999).

Biotransformation is any alteration of the molecular or atomic structure of a compound by microorganisms. Biodegradation is the breaking down of organic substances by microorganisms into smaller organic or inorganic components. These transforming and degrading processes occur as a result of microorganisms using the contaminants as a source of nutrients or energy, changing them through various metabolic reactions. Bioremediation depends on the presence of the appropriate microorganisms in the correct amounts and combinations and on the appropriate environmental conditions. Optimum environments for growth of microbes typically consist of temperatures ranging between 15°C and 45°C; pH values between 5.5 and 8.5; nutrient ratios (C:N:P) of 120:10:1. Atmospheric composition and water content may also influence microbial growth and activity. In addition, the contaminants must be in close enough proximity to the microbes and in a form that the microbes can utilize.

There are a number of *ex situ* and *in situ* bioremediation methods currently available (McCullough *et al.*, 1999; Romantschuk *et al.*, 2000). *Ex situ* methods have been around longer and are better understood; they are easier to contain and control. However, *in situ* bioremediation has several advantages over *ex situ* techniques. It offers a way of treating contaminants that are widely dispersed in the environment, present in dilute concentrations, or are otherwise inaccessible. It is more cost effective than *ex situ* techniques because no pumping or excavation is required. Also, *in situ* bioremediation may be less hazardous, as there is no exposure to the contaminant during treatment

Within the spectrum of bioremediation techniques, most techniques can be characterized as either engineered or intrinsic. Engineered bioremediation falls within two basic categories either biostimulation or bioaugmentation (Rittmann and Whiteman, 1994; McCullough *et al.*, 1999; Vogel, 1996). These engineered strategies can be used together or separately. Biostimulation is the addition of nutrients, oxygen, or other electron donors and acceptors to increase the number or activity of naturally occurring microorganisms available for bioremediation. These stimulants can be added in either liquid (soil washing) or gas (soil venting) forms. Bioaugmentation is a general term for adding specialized microorganisms or enzymes to the environment. It can be defined as the use of competent consortia or characterized strains of microorganisms with the abilities to degrade or transform the target toxic compounds. Bioaugmentation is usually used to improve a particular aspect of process performance. *Ex situ* bioaugmentation is a common technology at municipal wastewater treatment facilities. Commercial inoculants of enriched cultures consisting of one or more microbial species have been successfully used to colonize new

trickling bed filter systems and to rapidly recolonize systems where the intrinsic microbial community was victim to a system upset.

Researches is also beginning to focus on genetically modified microorganisms (GMMs) for use in bioaugmentation. *In situ* bioaugmentation with GMMs is still in the preliminary testing phase in fully or partly contained systems. There is a great deal of interest in the *in situ* use of GMMs for the treatment of hazardous wastes. Organisms with enhanced capabilities to degrade hydrocarbons, aromatic compounds, and halogenated compounds have already been developed. The intentional release GMMs, for use in cleaning up contaminated environments will move recombinant biotechnology from the laboratory into the field. However, concern about the deliberate release of GMMs to the environment has initiated international discussions regarding risk assessment. The safety aspects of intentional release were focused by OECD in 1986 and in 1992. The first OECD workshop on monitoring of organisms introduced into the environment was held in 1990, with a follow-up in 1992. At the United Nations Conference on Environment and Development in Rio, June 1992, a decision was made to „apply biotechnologies and their products to protect environmental integrity with a view to long-term ecological security" including GMMs and potential environmental impact. A biotechnology risk assessment research program was launched in 1986 by the US Environmental Protection Agency (Gustafsson and Jansson, 1993).

Intrinsic bioremediation occurs *in situ* and relies on the already-existing naturally occurring biological processes. Intrinsic bioremediation is a type of natural attenuation. Intrinsic bioremediation was first noticed a number of years ago at sites of petroleum hydrocarbon contamination. The pollutants were being biodegraded by the naturally occurring microorganisms at rates fast enough to stop or reduce contaminant spread. In order to establish that intrinsic bioremediation is actually occurring at these rates, plume size and metabolic activity must be measured over a period of time (McCullough *et al.*, 1999).

Microorganisms and molecular techniques in bioremediation

Bioremediation relies on the pollutant-degrading or transforming capabilities of microorganisms being utilized. The strategy is dependent on the catabolic activities of the employed microorganisms, optimizing the conditions for *in situ* growth and biodegradation. In many commercial bioremediation strategies the microbial consortia utilized are treated as „black boxes" without analyzing the constituent microbial populations and trying to understand how they actually function or if they may have critical syntrophic relationships. One of the problems that plagues scientists in bioremediation is how to identify and characterize the microbial communities that live at a contaminated site. Traditionally microbiological studies of pollutant-degrading microbes involve isolation, classification and physiological characterization. Culturing is a traditional method of identifying a microbial species. Microbial strains representing a single species is isolated from a mixed culture and grown in a sterilized

medium in a temperature-controlled incubator. Culture-dependent techniques, such as Biolog-generated community level physiological profiles (CLPP) are also used to estimate the *ex situ* metabolic potential of members of the microbial community isolated from a variety of environments. CLPP provides an indication of the metabolic diversity present in an environment with respect to the number of defined substrates that can be metabolized (Juck *et al.*, 2000). Such studies fail however, to reflect the true microbial diversity and activities occurring. Indeed, less than 1% of all microorganisms have been or can be cultured in the laboratory (Trevors, 1997; Watanabe and Baker, 2000). Microbial consortia involved in environmental biotechnology, *e.g.* activated sludge and soil/sediment consortia, are very complex, enabling them to act on a variety of pollutants. In the early 1990s, when good molecular biological techniques were developed to study microbial ecology researchers started to analyze microbial populations relevant to pollutant degradation in the environment (environmentally relevant microorganisms – ERMs) (Watanabe and Baker, 2000). Thus began studies of microbial consortia using molecular techniques, such as direct DNA isolation from environmental samples and after amplification determining the sequences of specific genes. After identification, the sequence can be compared to a large database comprising 16S rRNA, sequences of previously isolated organisms. Figure 1 summaries the main steps in the characterization of microbial consortia in environmental samples by using molecular techniques, but Figure 2 presents results using these molecular methods to determine the phylogenetic relationship of hydrocarbon-degrading bacterial community.

To analyse microbial communities several combinations of molecular techniques are used, such as: denaturing gradient gel electrophoresis (DGGE), PCR, TRFLP, nucleic acid hybridization (DNA-DNA, DNA-RNA, RNA-RNA) *etc.* (Hazen and Jimenez, 1988; Harry *et al.*, 2000). These techniques have advantages over traditional methods which lack the specificity and sensitivity required for bioremediation monitoring. Methods based on isolation and identification of the nucleic acids of target organisms overcome these problems. They have significant potential for detecting and monitoring the frequency maintenance and dispersal of natural microorganisms and microorganisms released into the environment (Atlas *et al.*, 1992). The combination of culture-dependent (traditional plating methods) and culture-independent methods both biochemical and molecular techniques provide a very sensitive way to establish microbial diversity in environmental samples. The high specificity, sensitivity and reproducible consistency of PCR detection of specific DNA sequences in complex environmental samples has contributed significantly to the advancement of knowledge in environmental microbiology (Holben and Tjedje, 1998; Holben *et al.*, 1988; Pickup, 1991; Bej *et al.*, 1991; Bej and Mahbubani, 1992; Amann *et al.*, 1995; Onuki *et al.*, 2000)

The goal of the environmental scientists may not only be to prevent further contamination, but also to clean up highly polluted areas to avoid migration of contaminants to ground and/or surface water.

DNA extraction Direct soil DNA extraction techniques are now an important part of microbial ecology investigations. They are very useful for determining the

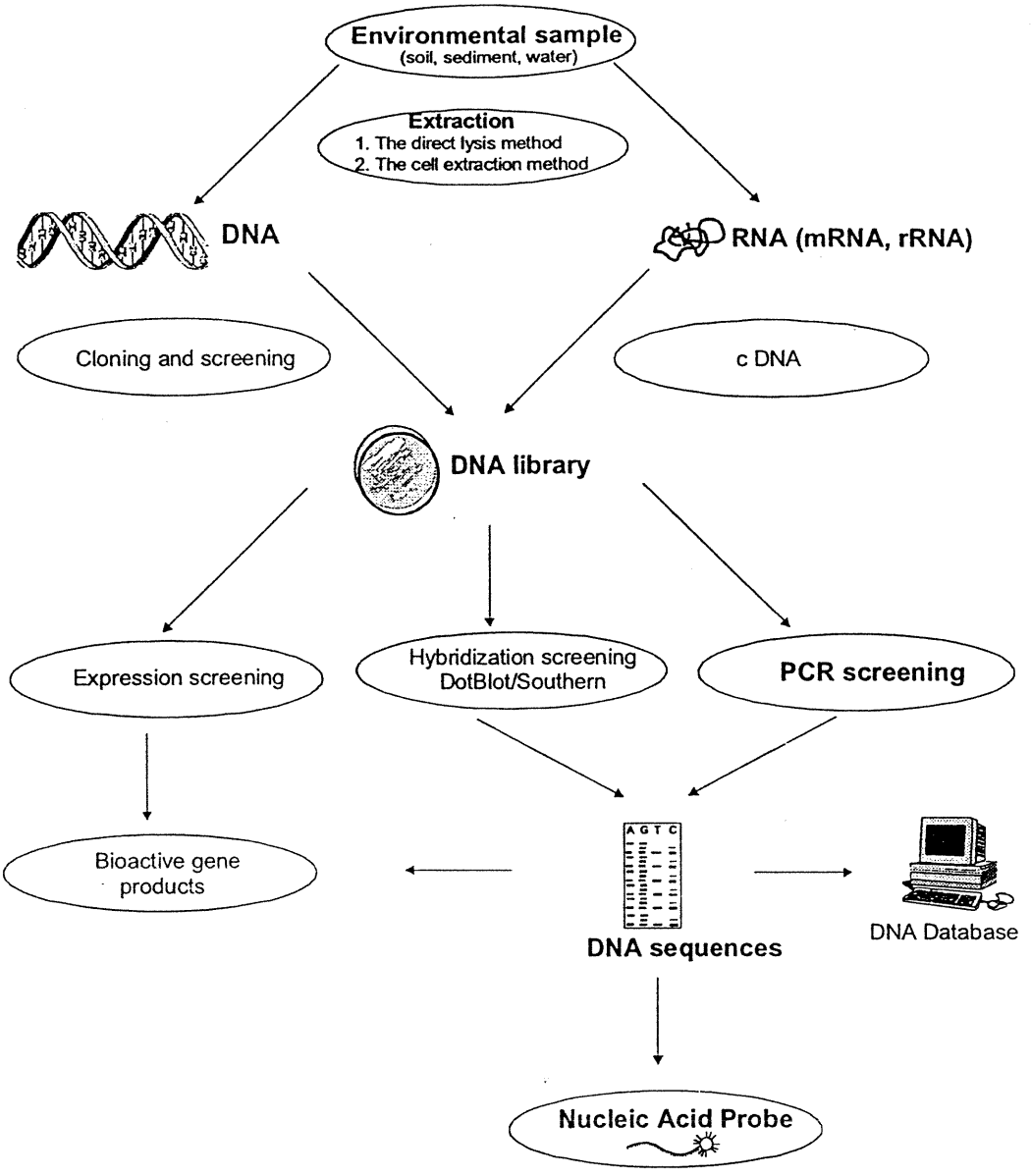

Fig. 1. Characterization of microbial biota in environmental samples by using molecular techniques (modified from Sayler and Layton, 1990; Amann *et al.*, 1995; Cowan, 2000)

presence of native bacteria or bacteria introduced into environments, GMOs or GEMs – genetically modified (engineered) organisms (Torsvik, 1980; Steffan *et al.*, 1988; Sommerville *et al.*, 1989; Tsai and Olson, 1991; Saano and Lindstrom, 1995). A large fraction, often 90–99% of microbial cells present in environmental samples are not culturable on microbiological media. Viable microbial-cells can be only partially recovered from complex environmental samples by traditional plating methods. Their detection *via* molecular techniques is often required to have a true understanding of the microbial ecology of any environment.

Two different techniques for isolation of DNA from soil can be carried out:

- the cell extraction method and subsequent lysis, or
- the direct lysis method

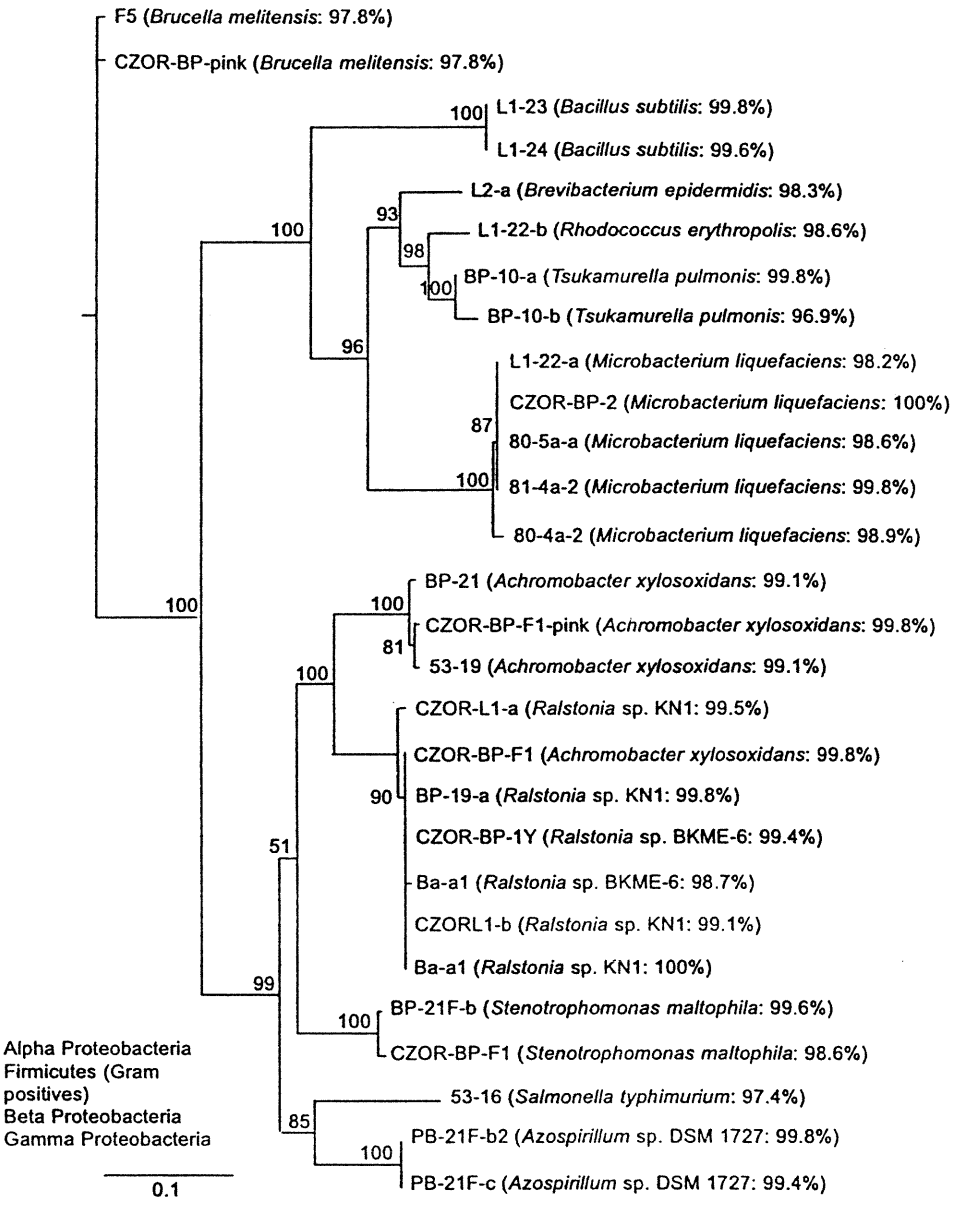

Fig. 2. Phylogenetic relationship of bacterial strains isolated from hydrocarboncontaminated soil (result of common work of Savannah River Technology Center, USA and IETU, Poland)

In the first technique, the extraction of microbial cells from soil precedes the DNA extraction, whereas in the second technique DNA is extracted directly from soil. Most methods described are based on direct extraction of DNA from water environments and from soil and sediment (Ogram *et al.*, 1987; Atlas, 1992; Saano and Lindstrom, 1995; Zhou *et al.*, 1996). The methods aim at the same goal: the highest yield of extracted DNA sufficiently pure to allow the identification using molecular techniques. The quantity of extracted and purified DNA can be assessed on agarose gels and compared with DNA markers. DNA concentrations are expressed as nanogram per gram of dry soil/sediment or nanogram per millilitres of water (Pickup, 1991). The direct lysis technique has the advantage of recovering DNA from organisms that are strongly sorbed and poorly removed by the cell extraction

methods. The direct lysis technique has the disadvantage of humic and pH interferences from the soil or water being extracted.

All methods aim at getting a high yield of DNA that is pure enough for molecular analysis by the DNA-DNA hybridization, the terminal restriction fragment length polymorphism (TRFLP) analysis or the amplification by the polymerase chain reaction (PCR). Humic and clay compounds in many soil samples inhibit the analysis, and the presence of colloids render the extraction of pure DNA problematic. For this reason an extensive purification step in the DNA isolation protocols is necessary (Sambrook *et al.*, 1989; Dijkmans *et al.*, 1993; Volossionk *et al.*, 1995).

Typically DNA isolation involves the following steps (Sambrook *et al.*, 1989; Pickup, 1991): 1. the lysis of the cells; 2. the separation of DNA from the cell components such as polysaccharides and proteins; 3. purification of the DNA extract from soil particles and components like humic acids, clay, or iron *etc.*; 4. the precipitation of DNA.

As reported ~ 300 ng DNA and ~100 ng RNA could be extracted from 10 g soil (Saano and Lindstrom, 1995). Some DNA recovery methods and their modification are shown in Table 1. Purification procedures can be any combination of CsCl-EtBr ultracentrifugation, hydroxylapatite or affinity chromatography, phenol/chloroform extractions, ethanol precipitation, dialysis, or repeated polyvinylpolypyrrolidone (PVPP) treatments (Sambrook *et al.*, 1989; Pickup, 1991). In many cases, standard purification protocols do not work with every environmental sample,

Table I
Isolation of DNA from environmental samples by various methods
(modified from Pickup, 1991)

Environmental samples	Sample size	Method of DNA extraction	Cell numbers per g	DNA yield
Water	> 1 litre	Direct cell lysis/ethanol precipitation or CsCl centrifugation	10^{6}*	1 ng
Soil	50 g	Cell lysis after dispersion and PVPP treatment/CsCl centrifugation	10^{6}	ND
Soil	100 g	Direct cell lysis/ethanol precipitation or CsCl centrifugation	10^{9}	350 µg
		Cell lysis after dispersion and PVPP treatment/CsCl centrifugation/hydroxyapatite chromatography/ethanol precipitation	10^{9}	40 µg
Sediment	100 g	Direct cell lysis/ethanol precipitation or CsCl centrifugation	10^{9}	1.9 µg
		Cell lysis after dispersion and PVPP treatment/CsCl centrifugation/hydroxyapatite chromatography/ethanol precipitation	10^{9}	30 µg
Sediment	100 g	Direct cell lysis incorporating glass beads/DNA precipitation/CsClcentrifugation/ethanol precipitation	10^{7}	2.6 µg

ND – not determined, PVPP – polyvinylopolypyrrolidone, * – cells per ml

and the required conditions must be adapted to an individual analysis. Several articles have reviewed recent progress in development of DNA isolation methods (Holben *et al.*, 1988; Sayler and Layton, 1990; Pickup, 1991).

PCR – polymerase chain reaction. The use of PCR in environmental microbiology was reviewed by Bej and Mahbubani (1992). The development of this technique was a major methodological discovery in molecular biology. The method is being used in every type of laboratory interested in molecular biology from diagnostics to pure research. The high specificity, sensitivity and reproducible consistency of PCR detection of specific DNA sequences in complex environmental samples has contributed significantly to the advancement of knowledge in environmental microbiology and many other areas of research (*e.g.* detection of microbial pathogens, clinical diagnostics, detection of mutation, generation of DNA probes by PCR, and the cloning of PCR products) (Pillai *et al.*, 1991; Toze, 2000; Watson and Blackwell, 2000). The most obvious application of this technique is to enhance gene probe detection of specific gene sequences. By amplification of a target sequence, PCR enhances the detection of rare sequences in complex mixtures of DNA isolated from environmental samples. The PCR is a powerful DNA amplification technique with the potential to provide a sensitive and specific method for monitoring microorganisms in the environment (Steffan and Atlas, 1991). A typical PCR process entails a number of cycles for amplifying a specific DNA sequence. Each cycle is composed of three stages: denaturation, reannealing, synthesis. One cycle generally lasts from 3 to 5 minutes. Each step during the single amplification is usually carried out in an automated, programmable block heater.

Generally, PCR involves repetitive cycling between a high temperature to thermally denature DNA, a relatively low temperature to allow the primers to hybridize (anneal) with the complementary region of the target DNA, and an intermediate temperature for primer extension or replication.

In environmental studies PCR method is used for detection of microorganisms, *e.g.*, genetically engineered microorganisms (GMOs or GEMs), pathogens, indicator organisms. Steffan and Atlas (1988) used PCR to amplify specific regions of a 1.0-kilobase (kb) length which was an integral portion of a larger 1.3-kb repeated sequence present in the genome of the herbicide-degrading bacteria *Pseudomonas cepacia* AC11000 to increase the sensitivity of dot-blot detection of the organism. Bacterial DNA was isolated from sediment samples. After amplification, *P. cepacia* AC1100 was positively detected at a concentration of 1 cell per gram of sediment. This represented a 10^3-fold increase in sensitivity compared with non-amplified samples. Chaudhry *et al.* (1989) also used PCR for detecting the genetically engineered microorganism – *Pennisetum purpureum*. Results of GMOs detection by using molecular techniques in environmental samples are presented in Jansson (1995) and Prosser (1994).

Historically, genes have been cloned from targeted organisms by generating a gene library of the genome investigated in phages like λ, M13mp, pUC or cosmide vectors, and then screening the library (usually in host cells – *E. coli*) for expression of the desired phenotype by selecting plating (Glick and Pasternak, 1988; Alberts

et al. 1999). PCR provides a relatively simple alternative to these procedures. PCR allows one to specifically amplify the region of DNA to be sequenced without developing gene libraries or performing extensive screening. This feature makes PCR particularly attractive for cloning and analyzing mutants of known genes, for cloning genes from different organisms, for subcloning genes or regions of genes where the nucleotide sequence is known, and even for isolating genes directly from natural, environmental samples.

Another very important use of PCR techniques is analysis of ribosomal RNA sequences for identification and phylogenetic characterization of microorganisms. This has been a major advancement in the study of microbial ecology. Phylogenetic identification and *in situ* detection of individual microbial cells without cultivation are presented in the review of Amann *et al.* (1995). The wealth of information presently available concerning highly conserved and variable regions within 5S and 16S rRNAs allows for relatively simple selection of primer target sites for amplifying desired rRNA gene sequences (Olsen *et al.*, 1986; Liu *et al.*, 1997; LaPara *et al.*, 2000). Manz *et al.* (1994) showed how specific oligonucleotides probes could be applied for the rapid *in situ* characterization of microbial communities in activated sludge of two wastewater treatment plants. In another paper, bacterial cells in soils and sediments were detected by PCR (Tsai and Olson, 1991). Jansson *et al.* (2000) summarize some of the developments in the use of biomarkers (or marker genes) which is defined as a DNA sequence introduced into an organism which confers a distinct genotype or phenotype for monitoring efficacy of bioremediation.

Holben *et al.* (1988) developed the use of sequence-specific DNA probes to detect specific genes and microorganisms in soil. Both a naturally occurring sequence, the *rbcL* gene, and sequence engineered into *Bradyrhizobium* strains, the *npt II* gene, were used as probes.

Next example using of molecular techniques in bioremediation process is characterization of the methanotrophic bacterial community present in a trichloroethylene–contaminated subsurface groundwater site described by Bowman *et al.* (1993). Contamination of subsurface environments with chlorinated hydrocarbons, in particular trichloroethylene (TCE) and tetrachloroethylene (PCE) is a potentially serious threat to drinking-water sources. The complete mineralization of TCE to CO_2 appears to be carried out most efficiently by the combined action of methanotrophic and heterotrophic microbial communities. Since metanotrophic bacteria are relatively ubiquitous in nature, they may serve as an instrument in the *in situ* bioremediation of contaminated sites. The distribution and characteristics of methanotrophic population involved in TCE degradation was done by DNA extraction and gene probes analysis (sMMO genes of enzyme methane monooxygenase) in environmental samples.

16S rRNA gene sequencing. This identification method can be used with *Archaea* as well as *Eukarya* and *Bacteria*. It is based on determining the phylogenetic position of the unknown microbe among known microorganisms. This determination is based upon a particular DNA strand – its 16S rRNA gene sequence. This sequence is considered the best for these evolutionary measurements because it is highly conserved among all species (Head *et al.*, 1998).

Obtaining the 16S rRNA sequence is accomplished in a variety of ways. One of the most common and effective is PCR, which replicates the 16S rRNA strand. This amplified material is then sequenced. Next, the sequenced 16S rRNA is compared to the sequences of other microorganisms that have been placed in a database (the Ribosome Database). Woese *et al.* (1990) have structured all three classes of organisms (*Archaea*, *Bacteria* and *Eukarya*) into relationships with one another based on the differences between the nucleotides in their 16S rRNA strand. Pairs of sequences from different organisms are aligned, and the differences in their nucleotide sequences are counted. The number of differences form a basis for measuring the evolutionary distance between organisms. In addition, knowing the phylogenetic position of an unknown, uncultured organism can sometimes allow inference of its physiological properties, which in turn can suggest culture conditions that allow its isolation (Olsen *et al.*, 1986; Amann *et al.*, 1995; Hazen, 1997; McCullough *et al.*, 1999).

Conclusions

Molecular methods can provide environmental microbiologists with a valuable set of unique tools to study microbial communities, functional capabilities of particular environments and specific biodegrader populations involved in bioremediation processes. DNA based methods have been demonstrated to be reliable methods for tracking many specific microbes and their DNA screenings could be applied as microbial activity indicators for a qualitative evaluation of contaminated soils, sediments, waters *etc.*, and bioremediation monitoring as shown in Table II.

The most popular technique in environmental research is the PCR method which is useful for (according to Bej *et al.*, 1992):

– tracking genetically modified microorganisms and monitoring indicator populations and pathogens in waters, soils, and sediments.

– measuring gene expression by viable microorganisms as well as detecting specific populations based upon diagnostic gene sequences.

– cloning genes, permitting sequencing of genes, even from environmentally important microorganisms that cannot yet be cultured.

The use of nucleic acids (DNA, RNA) for environmental assays is at a crossroads. Studies to date have clearly demonstrated the feasibility of developing a wide array of specific probes for organisms and genes of environmental interest. The environmental microbiologists should be able to apply the techniques for the detection and enumeration of microorganisms in the environment and for the study of gene transfer and maintenance in natural communities.

Molecular techniques have a great potential for an analysis of microbial diversity, but the results of these studies are still very fragmentary. By using these methods, new organisms can be identified, isolated, and characterized in respect to both phylogeny and physiology, in spite of a huge diversity within microbial communities. By these techniques, *in situ* monitoring can enable studies of microorganisms in their niches. This is very important for understanding of most rules and mechanisms of

Table II
Examples of bacterial DNA probes used for environmental studies (according to Hazen and Jimenez, 1988)

Target	Probe	Use	Sample
Tn5 mutants	Tn5	quantify	soil
Yersinia spp.	O plasmid	identification	water
Escherichia coli	ETEC	detection	water
Vibrio vulnificus	cytotoxin	identification	oyster
Vibrio cholerae	ETEC (LT)	identification	isolates
Salmonella spp.	chromosomes	detection	water
Bradyrhizobium japonicum	nptII	detection	soil
Legionella spp.	chromosomes	identification	water
Campylobacter spp.	chromosome	detection	water
Bacteroides thetaiotamicron	chromosome	detection	soil
Rhizobium spp.	chromosome	detection	soil
Pseudomonas fluorescens	rDNA	identification	soil
chitin degradation	chitobiase	functional	water
toulene degradation	TOL plasmid	functional	sediment
naphtol degradation	NAH-7 plasmid	functional	sediment
nitrogen fixation	nif	functional	sediment
mercury resistance	mer	functional	estuary
PCB degradation	4CB	functional	sediment

biogeochemical cycles which are usually catalyzed by consortia of microorganisms. These techniques will enable better control, monitoring and development of new bioremediation strategies that will provide cost effective, efficient and environmentally friendly methods for environmental cleanup.

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