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REVIEW ARTICLE

Genomic tools in bioremediation

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Abstract Bioremediation is a process that uses microorganisms or their enzymes to remove pollutants from the environment. Generally, bioremediation technologies can be classified as in situ or ex situ. In situ bioremediation involves treating the contaminated material at the site while ex situ involves the removal of the contaminated material to be treated elsewhere. Like so much else in biology, the ease and availability of genomic data has created a new level of understanding this system. Bioremediation capabilities of the microbial population can be analyzed; not only by physiological parameters, but also by the use of genomic tools, and efficient remediation strategies can be planned. PCR and DNA- or oligonucleotide-based microarray technology is a powerful functional genomics tool that allows researchers to view the physiology of a living cell from a comprehensive and dynamic molecular perspective. This paper explores the use of such tools in bioremediation process.

Keywords Bioremediation · Catabolic potential · Molecular tools · Metagenomics

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Introduction

Advances in technology and industrialization, bring with them, their unpleasant partners, pollution and degradation of the environment. The effects on the environment connected with industrial activities are mainly related to the production of industrial wastes. Management of these wastes is evolving with time and complexity of the problem. Because of the problems associated with pollutant treatment by conventional methods, such as chemical treatment, incineration or landfills, an alternative approach, bioremediation, or the use of organisms for the removal of contamination or pollutants has become a popular option [1]. Microbes, with their catabolic diversity, are the key players in biogeochemical cycles. Their major advantage is that microbiological processes are flexible; they adapt to variable conditions (self-regulation) and also to new molecules or combinations of new molecules [2]. Most of our knowledge of degradation by microbes has been gained from pure culture studies in the laboratory [3–5]. The basic limitation to this was the fact that the majority of the microbes cannot be cultured. In the last decade, however, molecular tools have facilitated the analysis of the natural microbial populations without cultivation [6]. The molecular approach of analyzing the 16S rRNA libraries gave more insight into the world of the microbial communities existing in a particular environmental niche. The phylogenetic approach defines the population of the microbial community, while the metabolizing capacity can be assessed by the use of the polymerase chain reaction (PCR) using specific designed primers from catabolic operons. Various genetic approaches have been developed and used to understand the metabolic pathways of organisms

relevant for biodegradation. New information on the metabolic routes opens the bottlenecks in the bioremediation process.

Bioremediation holds a great promise in clean-up of contaminated sites. However, the path from the lab into the field is riddled with problems that need to be addressed before this promise can be realized. Specifically, it is important to understand the inherent microbiota, their interaction with the environment, stress conditions and factors that will bring about the expression of their genotype. So, a multidisciplinary approach of microbiology, biochemistry, molecular biology, modeling and genomics is required to bring theory into reality.

Role of microbes/microbial community in bioremediation

One of the most important challenges of our time is to find and exploit existing biodegradative routes to eliminate pollutants from the environment. A large number of bacteria/biodegradation by sludge, have been reported for degradation of different industrial wastes, such as dyes [7], hydrocarbons, especially related to petrochemical wastes [8–10], tanneries [11], chlorinated aromatics [12], distillery [13], pesticides [14–18], heavy metals [19]. Similarly, there are phenomenon such as chemotaxis and its relevance in bioremediation has been reported using pure culture system in a model study [20]. The Energy Research Institute, New Delhi, demonstrated the application of carrier-based hydrocarbon-degrading bacterial consortium for bioremediation of crude oil contaminated agricultural lands in the north-eastern and western parts of India [10].

The application of genomic tools in identification of the microbial community has led to the discovery of unique bacteria that were not accessible by culture-based techniques. Nucleic acid extraction from target niches and amplification of the 16S rRNA gene by polymerase chain reaction (PCR) has proved extremely useful in assessing the microbial community [21, 22]. Changes in the population dynamics can also be assessed by analyzing the microbial diversity at different time points or after treatment/change in carbon source of the niche [23].

Cellular constituents popularly used in microbial analysis

Understanding the microbial community of an environmental niche using modern molecular techniques provides an exciting opportunity to overcome the drawbacks of the culture-based analysis and therefore greatly increases our understanding of microbial diversity and functionality in the environment. These new methods rely on the characterization of cellular constituents such as fatty acids, proteins and nucleic acids that can be extracted directly from environmental samples without the need for culturing and analysis of the molecular composition can be used to elucidate the composition of the microbial community [24, 25]. Phospholipids constitute an important fraction of the cell biomass and PLFA (phospholipids fatty acids analysis) have been used previously in the analysis of bacterial community [26]. The advent of gas-liquid chromatography (GLC) revolutionized the analysis of fatty acids and this technique is the most frequently used currently for microbial fingerprinting. Since every microorganism has its specific FAME (fatty acid methyl ester) profile, it can be used as a tool for microbial source tracking. Protein profiles are mainly used to understand functional families in the vast diversity. However, these are not without their drawbacks. Fatty acid composition can be influenced by temperature and nutrition and individual fatty acids cannot be used to represent specific species [27, 28]. The most popular cell constituent used in microbial analysis is the nucleic acid. DNA sequences provide the basis for our current classification of microbial species and most tools focus on the use of DNA for analyzing the microbial secrets.

Flow of genomics tools in bioremediation

Genomics tools can carry out an assessment of the available biological capability of any ecosystem using specific biosensors or biomarkers. Biosensors can monitor a biological output that can be converted into a measurable signal, for example, enzyme based biosensors can generate the signal either by product formation, the disappearance of substrate, or co-enzyme conversion [29]. Biomarkers are specified genotypes that can be used to track the survival and/or efficacy of specific bacteria in bioremediation. Examples of biomarkers include the luc gene, encoding firefly luciferase and the gfp gene, encoding the green fluorescent protein (GFP). The *luc* gene has been demonstrated to tag different bacteria used for bioremediation of gasoline or chlorophenols, where the bacteria were monitored on the basis of luciferase activity in cell extracts from soil [30].

In the process of bioremediation, different microbes render the key metabolic activities, which together set the rules for removal of pollutants. The community analysis of these microbes can be carried out using molecular tools such as denaturing gradient gel electrophoresis (DGGE), temperature gradient gel electrophoresis (TGGE), terminal restriction fragment length polymorphism (T-RFLP). DGGE analyses are employed for the separation of double-stranded DNA fragments that are identical in length, but differ in sequence. The technique exploits the difference

in stability of G-C pairing as opposed to A-T pairing. A mixture of DNA fragments obtained by PCR amplification are electrophoresed in an acrylamide gel containing a gradient of increasing DNA denaturants. DNA fragments richer in GC will be more stable and remain double-stranded until reaching higher denaturant concentrations. Double-stranded DNA fragments migrate better in the acrylamide gel, while denatured DNA molecules become effectively larger and slow down or stop in the gel. In this manner, DNA fragments of differing sequence can be separated in an acrylamide gel. Similarly the amplified DNA fragments can be separated on the basis of their melting temperature, or differentiated by restriction digestion. In the case of T-RFLP, the amplification is performed with one or both the primers having their 5' end labeled with a fluorescent molecule. The mixture of amplicons is then subjected to a restriction digestion, and then separated using either capillary or polyacrylamide electrophoresis. The general trend in community can be understood by using overall DNA profiling by DGGE/TGGE analysis [31] or T-RFLP [32], whereas, by constructing the PCR amplified 16S rDNA library, the specific of the community and its composition can be understood [6]. These communities share the carbon source viz, the pollutants and/or other organic molecules that are present in the ecosystem. The community structure is decided by the transformation and utilization rates for different organic molecules [33]. Other than the organic molecules, nitrogen and phosphorus also play a key role in survival of microbes in these harsh environments. To assess the possible metabolic network or key microbes present in these environments, the metagenome extracted from that sample is analyzed. The general approach used in the analysis is summarized in Fig. 1. Of the various tools used in metagenome analysis, the most relevant approach is time dependent analysis for target loci.

Tracking gene loci in the environment

The spectrum of organic molecules, natural and man-made, is very large, and so is the process of evolution in microbes to utilize this wide range of molecules. Naturally occurring microbial activities are the starting point for all biotechnological applications. It is therefore necessary to first gain knowledge of the metabolic capabilities of the microbial community in the target niche and to understand degradative pathways, biochemically and genetically, before we can formulate a successful bioremediation program. Bioremediation strategies rely on the catabolic capacities of microbes to transform harmful pollutants to harmless compounds [34]. Most of the inherent soil microbes posses catabolic enzymes which are capable of utilizing pollutants as their growth substrates. Microorganisms are the largest reservoir of genetic and biochemical diversity. They have been evolving for the last 3.8 billion years and inhabit virtually every environment, often thriving under extremes of nutrient concentration, pH, salinity, pressure, and temperature [35].

The biochemical potential of natural microflora can be assessed by monitoring the available catabolic gene pool using target catabolic genes or a target genotype can be tracked in environmental niches using PCR. For example, phenol degrading genotype was monitored in environmental samples by designing primers from the dmpN and pheA operons [36]. Moharikar et al. [37] have also demonstrated the tracking of catabolic gene loci *phe*B, *xyl*E, tod-isp, *bed*A and *nah*G responsible for the enzymes catechol 1,2-dioxygenase, catechol 2,3-dioxygenase, toluene dioxygenase-iron-sulphur protein component, benzene dioxygenase and naphthalene dioxygenase in activated biomass of effluent treatment plant treating wastewater generated at refineries. The natural degradative potential of a niche can be determined using tools like PCR or DNA hybridization, and bioremediation strategies can be planned for more effective degradation. If the target gene is present in low numbers, enrichment for degradative genotype can be an option [38–40], or in the absence of target gene, bioaugmentation can be an alternative, as demonstrated in treatment of wastewater containing hydrocarbons, where the bioaugmentation of the *alk*B genotype was shown to improve efficiency of treatment.

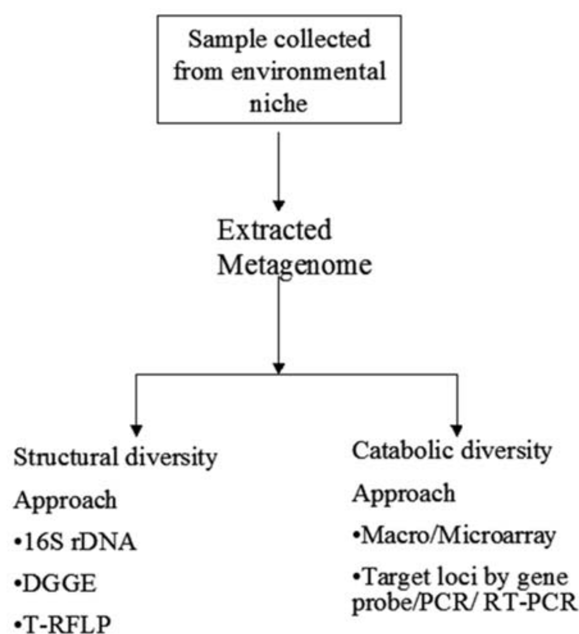

Fig. 1 General approach for analysis of metagenome in bioremediation

Monitoring the treatment of environmental pollution mainly involved the assessment of decrease of the pollutant at the contaminated niche. A new scenario is now emerging, where; a regular monitoring of different possible matrixes is becoming essential. In this process several monitoring stations are selected based on hydrogeology of that site. For example, it is not only essential to analyze the contaminated soil and leachate around the site, but, based on the possible movement of the contamination plume, analysis of the ground water also becomes mandatory. These analyses mostly include the assessment of the concentration of the target chemical or its possible metabolites. With the advent of molecular tools in bioremediation, it is possible to monitor the key genes are involved in conversion of a toxic intermediate to non toxic intermediate. Figure 2 describes an example for degradation of a hypothetical organic molecule in an environmental niche. Instead of monitoring only removal of the target compound, the genes involved in degradation may also be monitored to predict the efficiency of bioremediation. This analysis will let us know if the bioremediation program needs to follow the bioaugmentation or biostimulation route.

Isotope-labeled assays

Micro-autoradiography has been used for several decades to visualize and count cells within microbial communities using labeled substrates. However, the microorganisms being examined could not be identified. Fluorescence in situ hybridization (FISH) assays were developed to overcome this drawback. Target microorganisms on different phylogenetic levels could be detected by fluorescence microscopy using fluorescently labeled oligonucleotide probes. This technique has been reported in the determination of the structure and dynamics of microbial communities in environmental samples [41]. The method allows a specific staining and enumeration of soil microorganisms by using fluorescent-labeled oligonucleotide probes. However, the detection is often affected by strong autofluorescence of the background especially in soil samples. A number of variations to this tool are reported in literature [41, 42]. Catabolic potential of the microbial community has also been reported using isotope tracer methods. If a substrate of interest is labeled with an isotope tracer, organisms that uptake the substrate can be labeled in situ and traced using special detection tools [43].

Looking beyond the culturable microorganisms

A large number of bacterial strains have been reported in bioremediation by author's group [44–47], but it has been realized that to convert lab-scale research into technologies viable in environmental niches we need to look beyond culturable microorganisms. This is where gnomic tools come in. The bioremediation field has a lot to gain from the advances in this emerging area. Genomic tools are now used for elucidation of biodegradation pathways using PCR, DNA hybridization, Microarray analysis, isotope distribution analysis, molecular connectivity analysis, the assessment of mineralization process using metabolic footprinting analysis, and the improvement of the biodegradation process via metabolic engineering [48]. The future will also see the employment of various technologies including gene shuffling, high throughput screening, and nanotechnology [49].

Another important issue in microbial community analysis that needs to be understood in order to formulate a successful bioremediation program is the survival different gene pools in the contaminated niche. In these communities

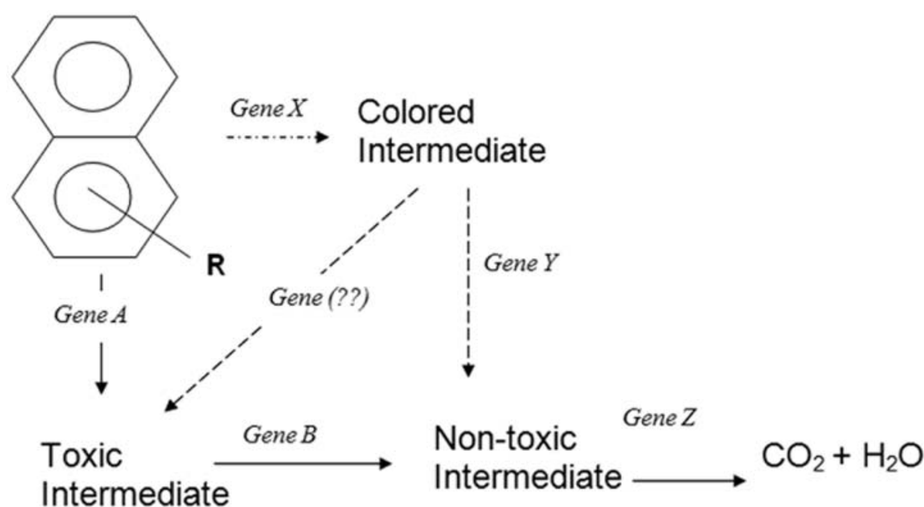

Fig. 2 Selection of target gene at different level of degradation for a hypothetical organic molecule

the primary biotransformation processes might start with a select group of bacteria that may not have the capacity to completely mineralize the molecule. These bacteria will only cause biotransformation and so will require different carbon sources for their growth. These can come from the degradation of other molecules in the contaminated niche and will generate a parallel pool of metabolites. The complexity of such carbon flow rules the community structure of the niche, which in turn will control bioremediation, and hence needs to be understood. Treatment strategies in such scenarios have been reported under controlled conditions. Analysis of microbial communities derived from these types of experiments carried out under controlled conditions, can give better understanding of survival of these communities. The efficiency performance of the biomass can be worked out by correlating the availability and expression of gene pool with the qualitative and quantitative analysis of organic molecules before and after treatment. To arrive at the maximum proficiency of biomass it can be further coupled with the different process operating parameters. The use of fuzzy logic in controlling such processes has been proved very effective in different studies [50, 51]. Parameters such as DO, NH_4 concentration, etc. could be defined linguistically and the fuzzy numbers could be generated for each linguistic term. A fuzzy rule-based system could be developed that incorporates the knowledge about the role of different parameters in deciding the assimilatory capacity of the treatment plant [52].

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