METABOLIC ENGINEERING AND FUTURE PROSPECTS IN BIOREMEDIATION: A MINIREVIEW

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ABSTRACT *Environmental pollution poses immense risk on health of living beings through organic and inorganic chemicals. Conventional techniques for decontaminating the environment were proven to be highly expensive and non-specific. Bioremediation is a promising method that uses microorganisms or their enzymes to promote degradation and/or removal of contaminants from the environment. Advancement in bioremediation techniques implies protein engineering tools for enzyme designing, molecular and genetic biology, novel metal sequestering peptides etc. as safe alternative approach compared to other physicochemical methodologies. Metabolic engineering are emerged from past decades to reveal its application in other fields like medicine, agriculture, xenobiotic metabolism and ecology. The present review discusses the recent advances in metabolic engineering for improved inorganic and organic chemical degradation using various tools.*

Keywords: *Environmental Pollution, Bioremediation, Protein engineering, Enzymes.*

Introduction:

Petroleum based products are the principle source of energy for day to day life but petroleum hydrocarbons is considered as toxic environmental pollutants [1]. Though, the unintended release of petroleum leads to widespread pollution of soil and aquifers, stimulating the need for an advancement in bioremediation processes. Bioremediation is a process in which microorganisms are used to detoxify and degrade the environmental pollutants [2]. Bioremediation is highly potential approach to clean up toxic environmental contaminants naturally yet inexpensively [3]. These microorganisms have catabolic genes which are able to synthesize metabolizing enzymes involved in hydrocarbon degradation [4]. A number of bacteria exhibits potential abilities in utilization of hydrocarbon substrates [5]. Human interventions for optimizing bioremediation techniques, including biostimulation of microbial communities, is essential to enhance the beneficial effects for a sustainable environment. Applications of metabolic engineering has been routinely applied for identification of millions of microbes through bimolecular and DNA engineering and their comparative approach for environmental monitoring [6].

In spite of all the advantages linked to enzymatic bioremediation, high production costs, low yields, and enzymatic inhibition are some of the problems that must be overcome. For that reason, molecular tools are being extensively explored to offer competitive enzymatic bioremediation products. Molecular tools allow us to detect genes related to degrading enzymes in environmental samples or isolates, thus serving as potent tools for bioprospection. This reviews deals with the archives of metabolic engineering and their future prospects in the field of bioremediation.

Biomolecular Engineering

Biomolecular engineering is a moderately new field of academic research and industrial practice with a goal of engineering biomolecules, such as proteins and nucleic acids, and biomolecular processes for biotechnological applications. Accelerating the evolutionary processes via biomolecular engineering techniques has become an increasingly attractive proposition for enhanced bioremediation strategies. The rational design approach for bioremediation typically involves either the construction of a single microorganism in which desirable biodegradation pathways or enzymes from different organisms are brought together to perform specific reactions using recombinant DNA technology (whole cell level), or the engineering of enzymes with desired characteristics using site-directed mutagenesis (protein level). Now, with the recent advances in biomolecular engineering, the prospect of short-circuiting the process of natural evolution to degrade environmental xenobiotic pollutants has been created. This has opened exciting new vistas for enhancing bioremediation programs in the coming years.

DNA Engineering

DNA engineering can considerably improve enzyme yield with lower costs [7]. In recent years, efforts have been made to create genetically engineered microorganisms (GEMs) to enhance bioremediation. This is done to overcome some of the limitations and problems in bioremediation. These problems are:

a) Sometimes the growth of microorganisms is inhibited or reduced by the xenobiotics.

b) No single naturally occurring microorganisms have the capability of degrading all the xenobiotics present in the environmental pollution.

c) The microbial degradation is a very slow process.

d) Sometimes certain xenobiotics are adsorbed on to the particulate matter of soil and thus become unavailable for microbial degradation.

Many PCR primers that target genes associated to petroleum-degrading enzymes, both in aerobic and anaerobic conditions, have already been described (Table 1). The utilization of these already-characterized primers may assist environmental screening of degrading abilities and may help to evaluate the potentials of microbial isolates. More primers can be described for specific pathways or to improve the comprehensiveness of known primers using available databases. Molecular tools also allow us to increase expression levels manipulating not only physiochemical conditions (optimal conditions), but also the organisms at a genetic level, to improve enzyme production in many different conditions, for instance, improving the efficiency and speed of the petroleum degradation, decreasing the time of the remediation process. Genetic manipulation would be also useful to allow or improve the petroleum degradation in extreme environments, such as cold or hypersaline sites [8].

Metagenomics

In metagenomics, total DNA is extracted from appropriately selected environmental samples, propagated in the laboratory by cloning techniques, submitted to sequence or function-based screenings and/or subjected to large-scale sequence analysis (Fig. 1). Functional screening of metagenomic libraries offer the advantage that it does not rely on sequence homology to known genes, and for this reason, has allowed the isolation of different enzyme classes from several environments. The probability (hit rate) of identifying a certain gene depends on multiple factors that are intrinsically linked to each other: the host–vector system, size of the target gene, its abundance in the source metagenome, the assay method, and the efficiency of heterologous gene expression in a surrogate host [9].

One of the first studies using metagenomics to study microbial degradation of aromatic compounds was performed by Suenaga and colleagues [10] who constructed a metagenomic library from activated sludge for industrial wastewater. The library was functionally screened for extradiol dioxygenase activities (enzymes for aromatic degradation) and 38 clones were subjected to sequencing analysis [11]. As a result, various types of gene subsets were identified that were not similar to the previously reported pathways performing complete degradation. Moreover, the authors discussed the fact that aromatic compounds in the environment may be degraded through the concerted action of various fragmented pathways. The organization of hydrocarbon degradation genes of selected metagenomic fosmid clones derived from a metagenomic library from Brazilian petroleum reservoir and functional screening for hydrocarbon degradation activities [12] The author found many putative proteins of different aerobic and anaerobic well described catabolic pathways. However, the complete catabolic pathways described for hydrocarbon degradation in previous studies were absent in the fosmid clones. Instead, the metagenomic fragments comprised genes belonging to different pathways, showing novel gene arrangements where hydrocarbon compounds were degraded through the concerted actions of these fragmented pathways. These results suggest that there are marked differences between the degradation genes found in microbial communities derived from enrichments of oil reservoir sample and those that have been previously identified in bacteria isolated from contaminated or pristine environments.

Metaproteomics

Proteome analysis has become a powerful tool for the investigation of global changes in prokaryotic gene expression that allows for high throughput screening of induced proteins as a function of environmental changes [14]. The term "proteomics" encompasses an expansive range of topics including: the identification, and quantification of proteins in cells, tissues and biological fluids; analysis of changes in protein expression under different conditions; characterization of post-translational modifications; and investigations into protein-protein interactions [15]. The data generated during proteomic investigations is precious to the research area of microbial biodegradation because it incorporates a comparative analysis of biodegradation

enzymes, it allows for the separation and identification of various post-translationally modified proteins, and allows for the analysis of open reading frames that are induced under certain conditions [16]. Important information on the life cycle, regulation and post-translational modification of proteins can also be provided by proteomic investigations [17]. Fig. 2 illustrates the basic outline of the workflow involved in a proteomic investigation.

The induction of proteins involved in biodegradative pathways is achieved through the growth of microbial cells on a variety of substrates available in an environment or culture medium [18] Various extraction techniques are available in the literature to recover desired proteins from the host cell; these may involve physical, chemical or enzymatic means to disintegrate the cell and commercial preparations for such tasks are available for almost any cell type [19]. Once isolated, the proteins are separated by electrophoresis on a 2-Dimensional polyacrylamide gel (2D-PAGE), a technique that is well established to allow for qualitative and quantitative comparisons of different protein expression profiles [20]. Proteomics provides a suitable platform to facilitate the study of the relevant pathways involved in the breakdown of hydrocarbons.

Proteomics in various microorganisms, particularly yeasts, and in human beings is already developed study [21]. Proteomics in fungus has been flourished in live areas during recent years [22]. Plant proteomics has also developed but likely to emphasized on just five species, even though plant science researchers are gaining confidence in this area [23]. Conversely, the area of soil proteomics has only just started and extensive technology and methodology has been made in recent years. However, the information obtained from the soil metaproteome could be of superlative importance for the reason that soil-mediated processes are related powerfully to issues of sustainability. In this sense, the importance of metaproteomics exists in the identification of proteins and microbial species implicated in soil processes, as indicators of soil eminence and functionality.

Unfortunately, there are no available studies that judge against the efficiency, in terms of yield and quality of extracted proteins present in soils, taking into account factors such as pH, buffer or temperature. Though, we should consider that pH is not only important because it influences the protein structure per se; modification may affect the adsorption of proteins to minerals, thus affecting the yield of the extraction method. Protein adsorption to minerals and organic matter is dependent on pH because proton activity controls both the physicochemical properties of the organic and inorganic adsorbents and, therefore, the extent of interaction [24]. As a model of protein adsorption on clays [25] showed that, at any pH above 4, a conformational change takes place within 10 minutes of contact between recombinant prion protein and montmorillonite particle surfaces.

Methods for extracting proteins should fulfil the subsequent requirements: (i) accomplish the quantitative extraction of proteins from the soil environment; (ii) Purification of extracted proteins, in order to abolish molecules that might hinder in the consequent analysis and (iii) the circumstances gone through in extraction analysis should not modify the protein structure, which would impede MS analysis. On the other hand, such a technique is difficult to achieve for soil. Proteins can denature or unfolded in order that their three-dimensional (3-D) structure is distorted but their primary structure remains integral. Various environmental factors like high temperature, acid or alkaline pH and a large ionic strength would alter the 3-D conformation of the protein. The interaction that stabilizes the three dimensional structures of these proteins gets weaker as they are highly sensitive to these factors [26]. The proteins extracted from soil further separated to acquire information on their expression profile. Enhancement occurred in the separation technology of proteins now days, but various methods have been already available before the introduction of 1D and 2D-SDS-PAGE [27]. Mass Spectrometry technique helped to study the composite ecosystems as soils. It provides information on the protein molecule, such as the mass of the peptide obtained from the protein molecule and its amino acid sequence hence it is transformed into tool for identification the proteins. Study of soil metaproteome also used to identify various enzymes which get affected affected by changes in soil conditions, because the alterations that occur at population level and in the expression of proteins are specific to the perturbation [28]. Other fields like human proteomics, microbiology and environmental science were get flourished by taking advantages through soil proteomics.

Conclusion:

Metabolic engineering is a promptly emerging field of research that has had an intense effect on the way the microbial world is viewed and studied. This new field of biology has proven to be rich and comprehensive and is making important contributions in many areas including ecology, biodiversity, bioremediation, bioprospection of natural products, and in medicine. This review has addressed in a coherent manner the diverse and multiple aspects of metabolic engineering and the multiplicity of the probable applications of the soil microbial communities. This area of study also provides a broad outlook of the use of metagenomics

and metaproteomics as a tool to recognize better the function and role of soil microorganisms. The purpose of this study therefore is to look at the protein expression patterns of various microbes and attempt to reveal mechanisms employed by these microorganisms in the breakdown of hydrocarbon substrates.

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Table 1: *Examples of modifying primers to amplify genes involved in petroleum degradation.*

Target	Function
Catechol 2,3-dioxygenasegenes	Degradation of aromatic compounds
ALKA and/or *ALKB* genes	Encode enzymes related to alkane degradation
bamA gene	Encodes 6-OCH-hydrolases (last step of the route of dearomatization of benzoyl-CoA)
bssA gene	Encodes the α subunit of benzyl succinate synthase (BSS), which starts the anaerobic degradation of toluene and xylene

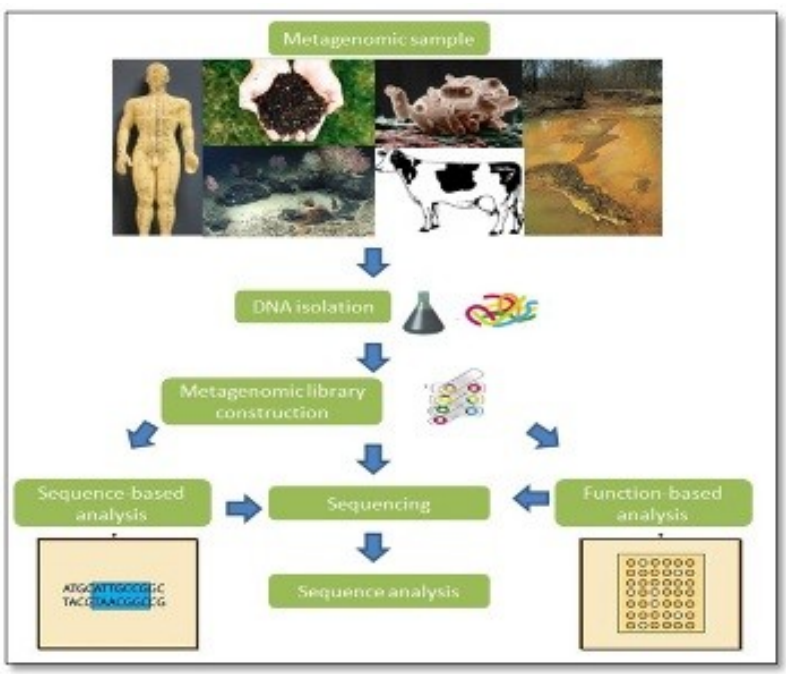

Figure 1: Schematic representation of the different steps of metagenomic analysis [13]

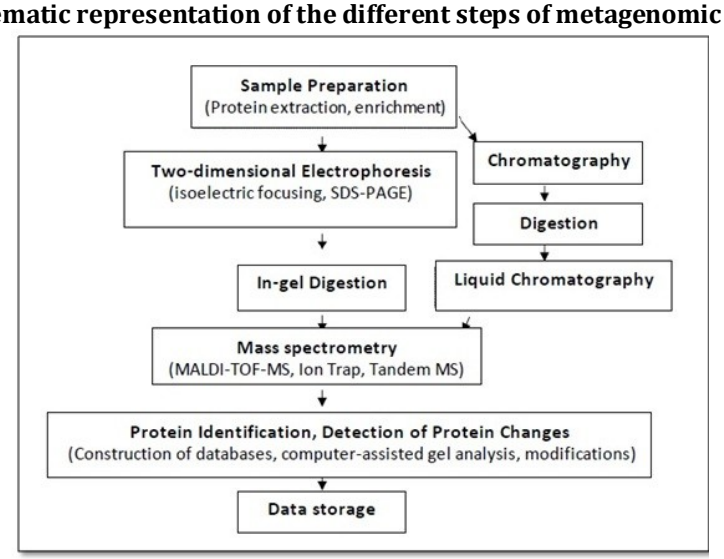

Figure 2 Basic workflow inveolved in proteomic investigations [17]