MOLECULAR BIOLOGY TECHNIQUES INVOLVED IN ENVIRONMENTAL MONITORING

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Abstract. A short review of the main molecular methods recently involved in monitoring environmental pollution is the aim of our aper. The elimination of a wide range of pollutants and wastes from the environment is an absolute requirement to promote a sustainable development of our society with low environmental impact. New methodological breakthroughs in sequencing, genomics, proteomics, bioinformatics and imaging are producing vast amounts of information. Functional genomic and metagenomic approaches will increase our understanding of the relative importance of different pathways and regulatory networks to carbon flux in particular environments and for particular compounds, and they will certainly accelerate the development of bioremediation technologies and biotransformation processes. Tools for in situ monitoring of environmental pollutants are destined for both aims providing early warning systems and conceiving more efficient and less expensive remediation treatments.

INTRODUCTION

Efficient tools for on-line and in situ monitoring of environmental pollutants are required to provide early warning systems. In addition, such tools can contribute important information on the progress of various remediation treatments. The application of biological sciences generally, and molecular biology techniques particularly, in monitoring environmental pollution has become increasingly prominent simultaneously with development of the DNA technology techniques. Interest in the microbial biodegradation of pollutants has intensified in recent years as mankind strives to find sustainable ways to cleanup contaminated environments. These bioremediation and biotransformation methods endeavour to harness naturally occurring, microbial catabolic diversity to degrade, transform or accumulate a huge range of compounds including hydrocarbons (e.g. oil), polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs), pharmaceutical substances, radionuclides and metals. Major methodological breakthroughs in recent years have enabled detailed genomic, metagenomic, proteomic, bioinformatic and other high-throughput analyses of environmentally relevant microorganisms providing unprecedented insights into key biodegradative pathways and the ability of organisms to adapt to changing environmental conditions.

The main biology techniques include: aerobic and anaerobic biodegradation of aromatic compounds, molecular detection methods (e.g. microautoradiography, mRNA analyses, etc.), genome-based predictive modelling, elucidation of regulatory networks, bioavailability, chemotaxis and transport issues, functional genomic analyses, natural attenuation, community fingerprinting and metagenomics, biotreatment, and biocatalysts engineering.

The bacterial genomic data provides opportunities for understanding the genetic and molecular bases of the degradation of organic pollutants. Recent genomic studies of
**Burkholderia xenovorans** LB400 [2] and *Rhodococcus* sp. strain RHA1 [3], two of the largest bacterial genomes completely sequenced to date, provide information concerning the aerobic degradation of aromatic compounds.

Anaerobic microbial mineralization of recalcitrant organic pollutants is of great environmental significance. In particular, hydrocarbons and halogenated compounds have long been doubted to be degradable in the absence of oxygen, but the isolation of hitherto unknown anaerobic hydrocarbon-degrading and reductively dehalogenating bacteria during the last decades provided ultimate proof for these processes in nature. With the increasing application of genomics in the field of environmental microbiology, a new and promising perspective is now at hand to obtain molecular insights into these new metabolic properties. Several complete genome sequences were determined during the last few years from bacteria capable of anaerobic organic pollutant degradation. The ~4.7 Mb genome of the facultative denitrifying "*Aromatoleum aromaticum*" strain EbN1 [4] was the first to be determined for an anaerobic hydrocarbon degrader (using toluene or ethylbenzene as substrates). The genome sequence revealed about two dozen gene clusters (including several paralogs) coding for a complex catabolic network for anaerobic and aerobic degradation of aromatic compounds. The genome sequence forms the basis for current detailed studies on regulation of pathways and enzyme structures. Further genomes of anaerobic hydrocarbon degrading bacteria were recently completed for the iron-reducing species [6] *Geobacter metallireducens* (accession nr. NC_007517) and the perchlorate-reducing "*Dechloromonas aromatica*" (accession nr. NC_007298), but these are not yet evaluated in formal publications [7]. Complete genomes were also determined for bacteria capable of anaerobic degradation of halogenated hydrocarbons by halorespiration: the ~1.4 Mb genomes of *Dehalococcoides ethenogenes* strain 195 and *Dehalococcoides* sp. strain CBDB1 and the ~5.7 Mb genome of *Desulfitobacterium hafniense* strain Y51 [8]. Moreover, genome sequences provided unprecedented insights into the evolution of reductive dehalogenation and differing strategies for niche adaptation.

The bioremediation process in subsurface environments may be opportune using extracellular electron transfer. Therefore, a systems biology approach to understanding and optimizing bioremediation with *Geobacter* species has been initiated with the ultimate goal of developing *in silico* models that can predict the growth and metabolism of *Geobacter* species under a diversity of subsurface conditions. To date, these studies have included sequencing the genomes of multiple *Geobacter* species and detailed functional genomic/physiological studies on one species, *Geobacter sulfurreducens*. Genome-based models of several *Geobacter* species that are able to predict physiological responses under different environmental conditions are now available. Quantitative analysis of gene transcript levels during *in situ* uranium bioremediation has demonstrated that it is possible to track *in situ* rates of metabolism and the *in situ* metabolic state of *Geobacter* in the subsurface. Initial attempts to link *in silico* *Geobacter* models with existing subsurface hydrological and geochemical models are underway. It is expected that this systems approach to bioremediation with *Geobacter* will provide the opportunity to evaluate multiple *Geobacter*-catalyzed bioremediation strategies *in silico* prior to field implementation, thus providing substantial savings when initiating large-scale *in situ* bioremediation projects for groundwater polluted with uranium and/or organic contaminants.

The two elements needed for an efficient utilization of aromatic compounds by bacteria are the enzymes responsible for their degradation and the regulatory elements that control the expression of the catabolic operons to ensure the more efficient output depending on the presence/absence of the aromatic compounds or alternative environmental signals.
Transcriptional regulation seems to be the more common and/or most studied mechanism of expression of catabolic clusters, although post-transcriptional control also plays an important role. Transcription is dependent on specific regulators that channel the information between specific signals and the target gene(s). A more complex network of signals connects the metabolic and the energetic status of the cell to the expression of particular catabolic clusters, overimposing the specific transcriptional regulatory control. In general, the regulatory networks that control the operons involved in the catabolism of aromatic compounds are endowed with an extraordinary degree of plasticity and adaptability. Elucidating such regulatory networks will pave the way for a better understanding of the regulatory intricacies that control microbial biodegradation of aromatic compounds, which are key issues that should be taken into account for the rational design of more efficient recombinant biodegraders, bacterial biosensors, and biocatalysts for modern green chemistry.

Organic solvents are toxic for microorganisms because they dissolve in the cytoplasmic membranes, a process that alters the membrane's physical structure and renders the cell unable to synthesize ATP. Genomic analyses in Gram-negative bacteria have revealed that the resistance-nodulation-cell division (RND) family of efflux pumps is the main group involved in the removal of solvents from the cell. These pumps are made up of three components that span the membranes and extrude solvents from the inner membrane or cytoplasm to the outer medium. The level of expression of these extrusion pumps is finely modulated by transcriptional regulators belonging to different families, but which act in a similar fashion. Some regulators act as repressors that prevent access of the RNA polymerase to the promoter region of the cognate operon. These regulators recognize multiple drugs through a series of overlapping binding pockets, and upon the binding of an effector, transmit a signal to the DNA binding region so that the regulator is released and the genes encoding the efflux pumps are transcribed.

Sustainable development requires the promotion of environmental management and a constant search for new technologies to treat vast quantities of wastes generated by increasing anthropogenic activities. Biotreatment, the processing of wastes using living organisms, is an environmentally friendly, relatively simple and cost-effective alternative to physico-chemical clean-up options. To perform a correct assessment, it is necessary to consider various microorganisms having a variety of genomes and expressed transcripts and proteins. In addition, a great number of analyses are often required.

The study of the fate of persistent organic chemicals in the environment has revealed a large reservoir of enzymatic reactions with a large potential in preparative organic synthesis, which has already been exploited for a number of oxygenases on pilot and even on industrial scale. Novel catalysts can be obtained from metagenomic libraries and DNA-sequence based approaches. Our increasing capabilities in adapting the catalysts to specific reactions and process requirements by rational and random mutagenesis broadens the scope for application in the fine chemical industry, but also in the field of biodegradation. In many cases, these catalysts need to exploited in whole cell bioconversions or in fermentations, calling for system-wide approaches to understanding strain physiology and metabolism and rational approaches to the engineering of whole cells as they are increasingly put forward in the area of systems biotechnology and synthetic biology.

Other recently developed environmental monitoring technologies involve the use of whole-cell biosensors [1, 5]. These biosensors are constructed through the fusion of promoters appropriately selected, responsive to the relevant environmental conditions, to easily monitored reporter genes. Their construction must be appropriate to detect general toxicity or specific toxicity caused by one or more pollutants. Currently, a large spectrum of microbial
biosensors have been developed that enable the monitoring of pollutants by measuring light, fluorescence, color or electric current. Depending on the choice of reporter gene, expression can be monitored by the production of colour, light, fluorescence or electrochemical reactions. Electrochemical monitoring is of special interest for in situ measurements as it can be performed using simple, compact and mobile equipment and is easily adaptable for on-line measurements. Recent advances in this area have included the development of biosensors of compact size that enable the on-line and in situ monitoring of a large number of environmental parameters.

CONCLUSIONS

1. The elimination of a wide range of pollutants and wastes from the environment is an absolute requirement to promote a sustainable development of our society with low environmental impact.
2. Bioindicators and biomarkers have the advantage that they measure the action of the pollutants in the real and complex environment where there may be many and complex interactions at sublethal levels.
3. New methodological breakthroughs in sequencing, genomics, proteomics, bioinformatics and imaging are producing vast amounts of information.
4. Functional genomic and metagenomic approaches will increase our understanding of the relative importance of different pathways and regulatory networks to carbon flux in particular environments and for particular compounds, and they will certainly accelerate the development of bioremediation technologies and biotransformation processes.
5. Whole-cell biosensors are finding increasing use for the detection of environmental pollution and toxicity.

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