Advances in monitoring of catabolic genes during bioremediation

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Abstract Biodegradation of xenobiotic compounds by microbes is exploited in the clean up of contaminated environments by bioremediation. Catabolic (or functional) genes encode for specific enzymes in catabolic pathways such as key enzymes in xenobiotic degradation pathways. By assessing the abundance or the expression of key genes in environmental samples one can get a potential measure of the degradation activity. One way of assessing the abundance and expression of specific catabolic genes is by analyzing the metagenomic DNA and RNA from environmental samples. Three major challenges in the detection and quantification of catabolic genes in bioremediation studies are 1) the accurate and sensitive quantification from environmental samples 2) the coverage of the enzymatic potential by the targeted genes 3) the validation of the correlation with actual observed degradation activities in field cases. New advances in realtime PCR, functional gene arrays and meta-transcriptomics have improved the applicability of catabolic gene assessment during bioremediation.

Keywords Catabolic genes · Functional genes · Bioremediation · Real-time PCR · Functional gene arrays · metagenomics; metatranscriptomics

Introduction

Biodegradation of xenobiotic compounds by microbes is exploited in the clean up of contaminated environments by bioremediation. Catabolic (or functional) genes encode for specific enzymes in catabolic pathways such as key enzymes in xenobiotic degradation pathways. By assessing the abundance or the expression of key genes in environmental samples one can get a potential measure of the degradation activity. One way of assessing the abundance and expression of specific catabolic genes is by analyzing the metagenomic DNA and RNA from environmental samples. This can be done by extraction of all DNA in e.g. a soil samples at different stages of a bioremediation action. The extracted DNA can then be exposed to a single gene PCR assay or it can be probed with sequences of known DNA e.g. plotted onto an array on a microchip for multiple gene assessment. The preparation of probes for functional genes requires existing sequence information, but on the other hand the search for new sequences of unknown functional genes can be obtained from cloning of metagenomic DNA, which again can be used as target on arrays. Three major challenges in the detection and quantification of catabolic genes in bioremediation studies are 1) the accurate and sensitive quantification from environmental samples 2) the coverage of the enzymatic potential by the targeted genes 3) the validation of the correlation with actual observed degradation activities in field cases.

Quantification of functional genes

A major progress in gene quantification is the development of specific real-time PCR methods. Whereas traditional PCR relies on the final end-point amplified product

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(presence or absence) the real-time PCR measures the amplified product through a fluorescent signal during the course of the amplification process (*ie* in real-time) and that allows for a back calculation of the original target copy number. Recent reports on the use and optimization of real-time PCR in actual bioremediation studies have mainly been concerned with bioremediation of contamination with naphthalene [1], nitro aromatics [2], arsenic [3] and for the anaerobic degradation of hydrocarbons [4]. The real-time PCR methods are more sensitive than older quantitative PCR methods such as MPN-PCR and RLD-PCR [5].

The coverage of enzymatic potential by single genes?

– The power of comprehensive arrays

The discovery of new biochemical reactions is small compared to the discovery of new genes from environmental samples [6]. Most described biochemical pathways are derived from isolated cultures. The rapidly increasing sequence data from metagenomic libraries and the use of bioinformatic tools has increased the number of known functional genes [7]. Furthermore a large number of genes encode for enzymes that catalyze the same reaction. Due to these constraints effort has been made to construct comprehensive microarrays that contain large numbers of DNA probes for the simultaneous probing of metagenomic DNA.

These functional gene arrays, also called FGAs, contain comprehensive gene probe sets. An array consisting of more than 2000 probes (50-mer oligonucleotides) from known genes involved in degradation processes was and used to assess functional genes in soil undergoing bioremediation [8]. This approach was extended by He *et al.*, (2007) [9] to produce the GeoChip, which contain probes for all currently known functional genes in the databases. This chip contains more than 20 000 probes targeting more than 150 functional groups of more than 10 000 genes involved in the biogeochemical cycling of nitrogen, carbon, phosphorous and sulfur along with metal resistance, metal reduction and organic contaminant degradation (Table 1). The probes are 50-mers and target gene sequences of both cultured organisms and sequences from metagenomic libraries. Probes involved in contaminant degradation comprise the biggest group with 6941 specific probes and 1087 group specific probes. The GeoChip was successfully used for assessing the microbial community differences in uranium bioremediation experiments. The GeoChip was also used for the detection of functional genes associated with aromatic degradation during a rhizoremediation experiment with PCB contaminated soil [10]. One of the main problems with the GeoChip is the sensitivity of the hybridization with metagenomic DNA, which has low abundance of many genes. The chip was only able to detect 8 organic con-

Table 1 Key properties of the comprehensive functional gene microarray, GeoChip 2.0 developed by He *et al.* 2007 [9]

Total number of genes >10 000	
Multiple probes for each gene	
Further information on probes and targets	http://ieg.ou.edu/GeoChip2.0/GeoChip2.0-info.xls
Categories of genes	Number of probes
Nitrogen cycling	5 310
Carbon cycling	4 599
Sulfate reduction	1 615
Phosphorous utilization	145
Metal reduction and resistance	4 546
Contaminant degradation	8 028
Total	24 243

Table 2 Future prospects for monitoring catabolic genes in bioremediation

More genes to be assayed
Use of full genome sequencing in the search of new catabolic genes
Real-time PCR and RT-PCR
Improvement of extraction for RNA
Sampling strategies from bioremediation events (different types of bioremediation)
Understanding by bioremediation performers, consultants and authorities
Databases
Standardization
Analytical services

taminant degradation genes from total community DNA. The hybridization with an enrichment of ^{13}C -SIP fractions revealed 28 organic degradation genes [10]. Methods to increase the concentration of target DNA has been developed by whole community genome amplification (WCGA) [11], where all the metagenomic DNA is evenly amplified to ensure representativeness of amplification.

Detecting expression of functional genes in environmental samples

The expression of functional genes in environmental samples can be assessed by the reverse transcription (RT) of whole community mRNA (metatranscriptomics), and this is of interest in bioremediation studies when assessing the actual activity of the gene on the site. This can both be done in context of a real-time PCR quantification (real-time RT-PCR) in single gene assays and in context of microarray assays where a large number of genes can be assayed at the same time. The major challenge in this approach is the low abundance and the labile nature of RNA. Transcriptomics have recently been applied in studies of the herbicide degrading gene *tdfA* where a success quantification of this gene was done in soil samples [12]. Recently a whole-community RNA amplification (WCRA) method was developed and applied before microarray analysis [13]. They were able to detect 39 genes from a uranium-contaminated groundwater sample from a site with biostimulation by ethanol feeding.

Comparison of functional gene abundances with actual degradation activity

Even though numerous studies on the detection and diversity of functional genes in environmental samples have been performed, rather few studies have actually thoroughly compared their findings to actual biodegradation rates from real bioremediation events [14, 15]. *NahAc* abundance and *nahAc* mRNA transcript levels were shown to correlate with naphthalene mineralization activity in PAH-contaminated soil in several studies [16, 17, 1], but for most other contaminants there is very little information on the correlation between the functional gene abundance and contaminant degradation activity.

Future prospects

The quantification of functional genes in the whole community genomic DNA and the inclusion of the diversity of functional genes into this, is a challenge for the development and the assessment of the efficacy of bioremediation technologies (Table 2). The search for new functional genes

from metagenomic libraries will continue [18] and comprehensive microarrays will need to be updated regularly [9]. The focus on assessment of bioremediation technologies relying on anaerobic processes such as monitored natural attenuation will increase [19, 15]. Real-time PCR will probably soon out compete older quantitative PCR methods due to the sensitivity and speed of the method. Today new methods such as real-time PCR and microarray assays are mainly used in research laboratories, but there may be an increased need for robust methods that can be standardized and commercialized in service laboratories due to the increased legislation on soil protection in many countries a growth in bioremediation businesses is to be foreseen.

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