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RESEARCH ARTICLE

SOIL METAGENOMICS: A PROSPECTIVE APPROACH FOR NOVEL ENZYME DISCOVERY

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ABSTRACT

The development of metagenomics has emerged as an alternative approach for the conventional screening of microbial genomes from the natural environment. Metagenomic analysis provides broad information on the structure, composition and gene functions of various environmental microbes. It has been successfully applied to screen novel enzymes from the uncultured microbes in the environment. The soil metagenomic approach relies on the direct isolation of environmental DNA. The main application depends on the construction of a metagenomic library in a suitable vector and host with subsequent high-throughput screening. This review focuses on the metagenomic approach for exploring novel enzyme discovery.

INTRODUCTION

Metagenomics can be defined as the genomic analysis of the collective microbial sequence found in an environmental sample (Handelsman *et al*., 1998). It is a culture-independent analysis of microbial genome communities. Soil is considered as a complex environment, which appears to be a major reservoir of microbial genetic diversity (Robe *et al*., 2003). Each organism in an environment has a unique set of genes in its genome and together forms the metagenome. Metagenomics has led to the accumulation of DNA sequences and these sequences are exploited for novel biotechnological applications (Ferrer *et al*., 2005a). There are many variants on metagenomic approaches which initially were dependent upon cloning of a DNA from an environmental sample (Healy *et al*., 1995, Stein *et al*., 1996), but more recently many metagenomic approaches have relied on high-throughput screening (Edwards *et al*., 2006). Metagenomics provides a determining route to find genes essential for making an industrial enzyme. Recently, several studies have applied a metagenomic approach to a number of different environments, such as soils (Rondon *et al*., 2000, Voget *et al*., 2003, Tringe *et al*., 2005), the complex micro-biome of the rumen (Brulc *et al*., 2009), planktonic marine microbes (Beja *et al*., 2000a, Breitbart *et al*., 2002), deep sea microbes (Sogin *et al*., 2006), an acid mine site (Tyson *et al*., 2004), arctic sediments (Jeon *et al*., 2009) and the Sargasso Sea (Venter *et al*.,2004). Metagenomes are screened based on their function or sequence. Function based-screening is a simple way of obtaining genes which has desired functions. Sequence-based screening is performed using either PCR-based or hybridization-based methods. Metagenomics have been advanced by the detection of essential ecological studies and focused screens for bio-prospecting and contributing to the discovery of enzymes from uncultured microorganisms. The promise of metagenomics involves the strategy for the identification of novel natural bio-active products, xenobiotic pathways and other metabolic processes, soils present a unique set of technical challenges for the successful isolation and analysis of metagenomic DNA. Much advancement in heterologous gene expression, library construction, vector design and screening may still improve it more; the current technology has proved to be sufficiently powerful for the discovery of enzymes.

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Approaches of metagenomic analysis

Soil is the major component of most terrestrial environments with diverse microbial population. The soil environment is an abundant yet under characterized source of genetic diversity that has great potential to enrich our understanding of soil microbial ecology and provide enzymes with bioactive compounds useful to human society (Kakirde *et al*., 2010). The physical composition of the soil will greatly influence its microbial population, as will its chemical characteristics such as organic matter content and pH (Hassink *et al*., 1993). Selecting a sampling site and its method is an important factor to consider for the metagenomic analysis of soil microorganism. Metagenomic employs multi-step process for the isolation of genetic material from environmental soil samples. The steps involved are isolation of DNA, manipulation of the genetic material, construction of metagenomic library and analysis of the genetic material in the metagenomic library. The first consideration for the extraction of the metagenomic DNA is the size of the sample. The DNA isolation can be broadly classified into direct and indirect extraction methods. Direct DNA isolation is based on cell lysis within the sample matrix and subsequent separation of DNA from the matrix and cell debris (Ogram *et al*., 1987).

It often results in the higher percentage of non-bacterial DNA (Ogram *et al*., 1987, Tsai and Olson 1991, Tebbe and Vahjen, 1993). The indirect approach involves the separation of cells from the soil matrix followed by cell lysis and DNA extraction (Holben *et al*., 1988). This extraction overcomes some limitations of the direct extraction methods because it results in less non-bacterial DNA (Osborn and Smith 2005). After cell lysis, deproteinisation in organic solvents, like phenol, phenol-chloroform, and chloroform-isoamyl alcohol are used before precipitating the metagenomic DNA (Ogram *et al*., 1987; Tsai and Olson 1991). Cloning large fragments of DNA isolated directly from the natural environment provides a method to assess soil metagenomic DNA. The size, complexity and diversity of the soil metagenome are examined by the large insert libraries. These libraries from the soil metagenome have been generated using cosmids (Entcheva *et al*., 2001, Courtois *et al*., 2003), bacterial artificial chromosome (BAC; Rondon *et al*., 2000) or fosmids (Quaiser *et al*., 2002, 2003). The choice of extraction and purification method depends on which cloning vector is used. When constructing a metagenomic library, size of the metagenome and cluster organization of genes has to be considered. Metagenomic libraries should contain clones with larger DNA inserts. Isolation of high molecular weight DNA provides the cloning of DNA into bacterial artificial chromosomes (BAC's) and allows the characterization of large regions of the genomes (Berry *et al*., 2003). They can provide significant advantages for some applications since they enable identification and characterization of intact functional pathways encoded on large contiguous DNA fragments (Stein *et al*., 1996, Beja *et al*., 2000b, Rondon *et al* 2000, Courtois *et al*., 2003).

Metagenomic Library screening technologies:

The analysis of metagenomic libraries involves two main strategies, function-based screening and sequence-based screening. They are generally used to screen and identify novel biocatalysts or genes involved in the production of antibiotic from metagenomic libraries.

Function – based screening

Function-based methods involve screening a metagenomic library to detect the expression of a particular phenotype conferred on the host by cloned DNA (Henne *et al*., 1999). This approach enables rapid detection of clones which has a potential application in industry. This method requires expression of the function of interest in the host cell. Functional analysis has identified novel antibiotics (Courtois *et al*., 2003; Gillespie *et al*., 2002), antibiotic resistance genes (Diaz Torres *et al*., 2003, Riesenfeld *et al*., 1999), degradative enzymes (Healy *et al*., 1995; Henne *et al*., 1999, 2000), detection of enzymes involved in poly-3 hydroxybutyrate metabolism (Wang *et al*., 2006), DNA polymerase 1 (Simon *et al*., 2009), operons for biotin biosynthesis (Entcheva *et al*., 2001), lysine racemases (Chen *et al*., 2009) glycerol dehydratases (Knietsch *et al*., 2003), naphthalene dioxygenase (Ono *et al*., 2007) and novel natural products. Another approach for functional screening of metagenomic libraries is to use host strains or mutants of host strains that require heterologous complementation for growth under selective conditions (Simon and Daniel, 2009). This screening can also be performed to detect a specific phenotypic characteristic, in which individual clones are assayed for a particular trait.

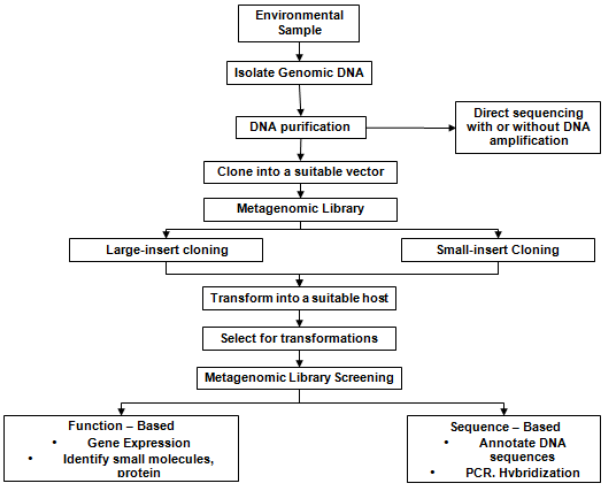

Fig. 1. Schematic representations of steps involved in the construction of Metagenomic Library

Sequence – based screening

Sequence-based screening involves direct sequencing of metagenomic DNA, either with or without cloning prior to sequencing and then subjecting the sequences to bioinformatics analyses (Kunin *et al*., 2008, Sleator *et al*., 2008). Sequence data analysis can consume more time and resources. Bioinformatics approaches are used to examine metagenomic sequence data sets. A useful tool for assessing metagenomic information is CAMERA (Community cyber-infrastructure for Advanced Marine Microbial Ecology Research and Analysis), developed to serve the needs of the microbial ecology research community by creating a data repository and a Bioinformatics resource to facilitate metagenomic sequence data storage, access, analysis and synthesis (Smarr, 2006). Bioinformatics tool for gene prediction such as, MEGAN (MEtaGenome ANalyzer), a program which compares a set of DNA reads against databases of known sequences using comparative tools such as BLAST algorithms. MEGAN can then be used to compute and interactively explore the taxonomical content of the dataset by using NCBI taxonomy to summarize and order the results (Huson *et al*., 2007). A freely available open source system that can process metagenome sequence data is the metagenomics RAST server (MG-RAST) (Meyer *et al*., 2008). Several novel industrial enzymes have been screened using sequence based analysis. Both the function and sequence-based screening strategies have been applied to isolate novel biocatalysts from metagenome, and their approaches are laborious due to the low frequency of clones with desired traits (Daniel *et al*, 2004). The Substrate Induced Gene Expression Screening (SIGEX) is an additional functional screening approach to identify genes for substrate catabolism and its utility was evaluated for the screening of aromatic hydrocarbon – induced genes from a ground water metagenome library (Henne *et al*, 2000).

Metagenomic applications in natural product discovery: Enzymes

High throughput screening of metagenomic DNA libraries are used for the discovery of many novel industrial enzymes. The

first metagenomic study involves in the identification of cellulases from a bioreactor 'zoolibrary' (Healy *et al*, 1995). A multifunctional glycosyl hydrolase identified from a rumen metagenomic library (Palackal *et al*, 2007), low pH, thermostable α-amylases discovered from deep sea and acidic soil environments (Richardson *et al*, 2002), agarases from soil (Voget *et al*, 2003), pectinolytic lyases from soil samples containing decaying plant material (Solbak *et al*, 2005) and lipolytic enzymes such as esterases and lipases (Rondon *et al*, 2000; Voget *et al*, 2003; Lee *et al*, 2004; Ferrer *et al*, 2005b). A novel β-glucosidase gene isolated by screening a metagenomic library derived from alkaline polluted soil was found to be a first member of a novel family of β-glucosidase genes (Jiang *et al*, 2009). The discovery of a diverse set of genes that encode enzymes for cellulose and xylan hydrolysis from the resident bacterial flora of the hindgut paunch of a wood-feeding 'higher' termite (*Nasutitermes sp.*) and from moths was a result of metagenomic analysis (Brennan *et al*, 2004; Warnecke *et al*, 2007). Mining for biocatalysts from metagenomic libraries usually involves three different strategies: i. homology – driven metagenome mining based on high-throughput sequencing, ii. Substrate-induced gene expression, iii. Function-based screening (Kakirde *et al*, 2010). The discovery of novel enzymes through these approaches is an economically responsible way to decrease the use of toxic chemicals in industrial applications.

Conclusion

Metagenomic approach can be used as a powerful tool to understand the various functions of microorganisms present in the native environment which can be applied in various biotechnological applications. The use of cutting-edge metagenomic based technologies to evaluate soil microbial communities has led to an extraordinary increase in the discovery of pathways that encode different gene products such as enzymes and anti-microbial compounds. The metagenomic strategies can improve the efficacy of existing methods and also enable the production of various chemicals that serve as precursors in various industrial applications.

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