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Current Opinion in Microbiology

Harnessing the power of microbial metabolism

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Microorganisms are rich repositories of genetic material encoding many activities of potential interest. Recent advances make identifying and exploiting the metabolic treasures of uncultured microbes an easier proposition. Improved expression vectors and metagenomic screening techniques make it easier to identify activities of interest. Synthetic biology and efficient genome editing techniques allow microbial genomes to be modified almost without restriction. Computational approaches based on organism-wide analysis of transcription, protein synthesis and metabolic fluxes make it possible to accurately predict the outcome of the metabolic processes and modifications required for optimization. Together these advances represent a major breakthrough in microbial biotechnology that is expected to yield new generations of tailor-made biocatalysts suitable for multiple biotechnological applications.

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Current Opinion in Microbiology 2016, **31**:63–69

This review comes from a themed issue on **Environmental microbiology**

Edited by **Monica Vasquez** and **Steven Hallam**

http://dx.doi.org/10.1016/j.mib.2016.03.003

1369-5274/Published by Elsevier Ltd.

Introduction

Microorganisms are the most abundant and diverse living creatures on Earth, have been able to colonize even the harshest natural environments and can utilize many different organic and inorganic molecules as sources of energy and nutrients. At the same time, microbes host a huge variety of enzymatic activities and metabolic pathways leading to the production of a plethora of primary and secondary metabolites. Many of these metabolic activities have been harnessed in industrial processes ranging from the food, chemical [1] and pharmaceutical sectors [2] to growing areas such as environmental bioremediation [3], biopesticide production [4] and bioenergy [5]. In this paper, we will review recent advances that improve our ability to exploit the metabolic potential of microbes. The review will look at the three main steps involved in efficiently harnessing microbial metabolisms: (i) identification of activities of interest and the genes encoding them, (ii) engineering optimized microbial biocatalysts by modifying coding genes and regulating their expression in a microbial host, and (iii) improving biocatalyst performance by integrating desired functions into the physiology of a single microorganism or complex microbial communities.

Identification of activities of interest

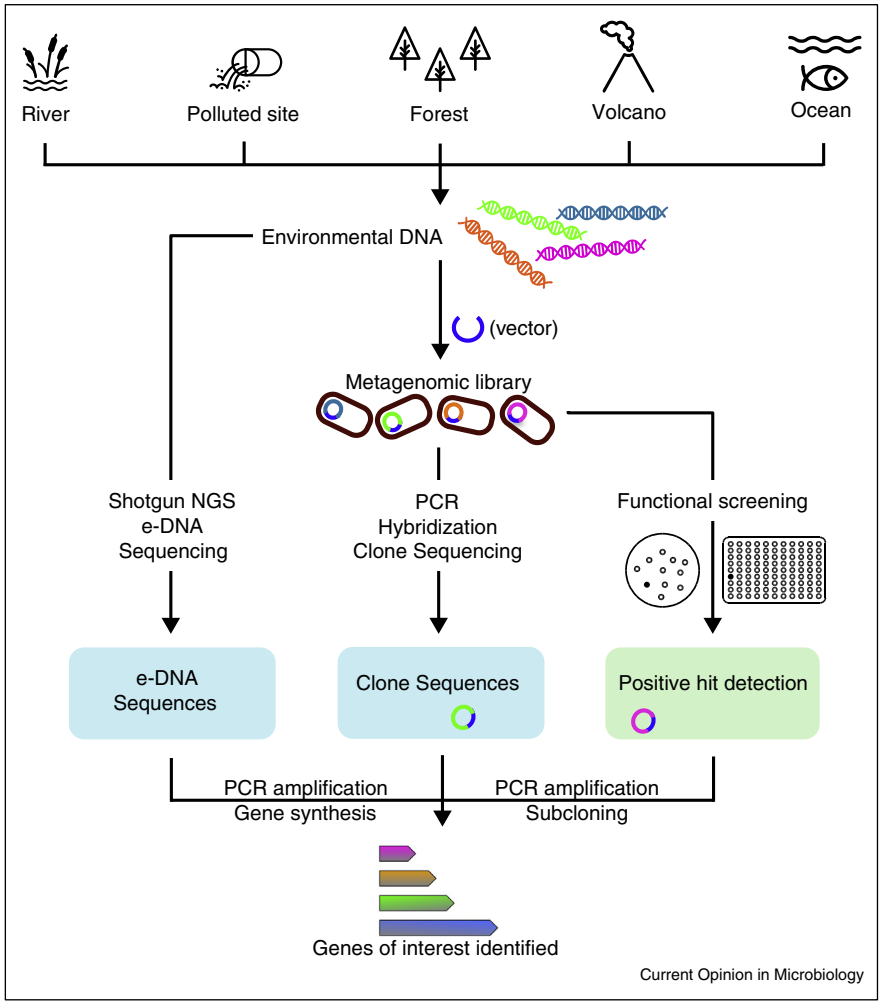
Most microbial activities and metabolic pathways used in industrial processes have been isolated from lab-cultured microorganisms. Designing screens for specific activities from lab grown microorganisms is relatively straightforward, but such screens are often inefficient due to redundant isolation of similar activities [6,7]. Cultured microorganisms represent less than 1% of the microorganisms thriving in nature. Enormous efforts are being made to cultivate different kinds of bacteria from complex habitats [8] but few have been successful in identifying new functions (for instance, [9•]).

A promising alternative is to use culture independent techniques such as metagenomics. Metagenomics involves directly isolating and analyzing microbial DNA from environmental samples and has been successfully used to discover many new enzyme activities [10]. Metagenomic analyses can be based on the functional screening of libraries constructed from environmental DNA or the similarity of metagenomic DNA to sequences with known structures or functions (Figure 1).

Functional screening relies on introducing metagenomic library clones into surrogate bacterial hosts and detecting phenotypic changes of interest. Its main advantage is that it does not require prior knowledge of the gene(s) encoding activities of interest, thus may lead to the discovery of activities encoded by genes lacking homologies to known sequences. A large number of novel enzymatic activities such as dioxygenases, esterases, proteases and nitrilases (recent discoveries in [10]), as well as lignocellulosic biomass depolymerizing activities [11,12] and the production of bioactive compounds [10,13] have been discovered by functional screening. However, functional analyses face two major challenges: poor gene expression in the surrogate host and a limited range of screening procedures for activities of interest [14].

Differences in the recognition sequences for gene expression make it unlikely that a gene from an unknown

Figure 1



Identification and cloning of genes of interest from the environment. Environmental DNA, isolated from a site of interest, can be used for shotgun sequencing or to construct metagenomic DNA libraries. Libraries can be screened for a desired function or for similar database sequences encoding the desired function. Genes coding for the desired function in the positive hits identified in the functional screening (green box), are identified by mutational analyses or subcloning. Genes of interest identified by sequence or function based screens of the metagenomic library can be subsequently subcloned into expression vectors. Genes of interest identified by shotgun sequencing or by mining DNA sequence databases are then obtained by PCR amplification from environmental DNA (e-DNA) or, if the DNA source is unavailable, by in vitro synthesis.

origin will be effectively expressed in a particular host. One solution is to construct the metagenomic library in vectors able to replicate in different bacteria, and then perform functional screens in as many surrogate hosts as possible. A number of broad host range vectors for gram-negative bacteria and shuttle vectors have been successfully used to screen metagenomic libraries in *Bacillus* [15], *Streptomyces* [16] and in thermophilic bacteria [17] (reviewed in [14]). An alternative approach is to drive the transcription of metagenomic DNA from phage promoters present in the vectors, which are less sensitive to transcription termination signals [18]. More recently, vectors have been developed whose transcription is subjected to antitermination by the lambda phage antitermination protein [19•]. Another successful approach has been to extend the *E. coli* promoter recognition capacity by expressing genes encoding the sigma transcription initiation factor from different bacteria [20••].

A diverse range of functional screens can be performed by direct colony screening on plates or by high throughput screening (HTS) of individual or pooled clones in microtiter plates [14]. Direct colony screening is straightforward but its sensitivity is low, the number of available assays is limited and is less useful for detecting intracellular activities or extracellular but non-secreted enzymes. On the other hand, HTS requires robotized handling procedures but allows cell permeabilization, enabling more diverse biochemical assays, and is better suited for large-scale screens. Multiplex systems have been