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Review

A review of metabolic and enzymatic engineering strategies for designing and optimizing performance of microbial cell factories

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ABSTRACT

Microbial cell factories (MCFs) are of considerable interest to convert low value renewable substrates to biofuels and high value chemicals. This review highlights the progress of computational models for the rational design of an MCF to produce a target bio-commodity. In particular, the rational design of an MCF involves: (i) product selection, (ii) de novo biosynthetic pathway identification (i.e., rational, heterologous, or artificial), (iii) MCF chassis selection, (iv) enzyme engineering of promiscuity to enable the formation of new products, and (v) metabolic engineering to ensure optimal use of the pathway by the MCF host. Computational tools such as (i) de novo biosynthetic pathway builders, (ii) docking, (iii) molecular dynamics (MD) and steered MD (SMD), and (iv) genome-scale metabolic flux modeling all play critical roles in the rational design of an MCF. Genome-scale metabolic flux models are of considerable use to the design process since they can reveal metabolic capabilities of MCF hosts. These can be used for host selection as well as optimizing precursors and cofactors of artificial de novo biosynthetic pathways. In addition, recent advances in genome-scale modeling have enabled the derivation of metabolic engineering strategies, which can be implemented using the genomic tools reviewed here as well.

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1. Introduction

In traditional chemical processes, a low-value starting material is converted into a high-value product through a series of unit operations. Initial operations may concentrate or refine the starting material by

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separating it from contaminants. The processed starting material is reacted with additional substrates in the presence of a catalyst, and the product of interest is separated from unreacted substrates and byproducts. Advances in catalysis and process optimization maximize single-pass conversion and profitability. Microbial cell factories (MCFs) have emerged as a revolutionary platform for combining traditional unit operations and complex multi-step catalysis into a single self-replicating microbe [1–3]. Reactors filled with billions of microbes can now replace much of the traditional chemical factory. Each cell can selectively uptake a low value substrate and use its vast metabolic network (and compartmentalization if necessary) to produce desired products. This review covers recent advances in (i) how chassis microbes are selected and engineered to serve as an MCF, (ii) how new catalytic properties are added to the metabolic network, and (iii) how the cell is engineered to use new metabolic pathways to maximize yield of a desired product. Methods are often grouped into combinatorial (i.e., evolutionary) and rational (i.e., informed design) approaches. This review specifically targets rational approaches that are informed by computational models and demonstrates how computational approaches are advancing the design of a complete, custom MCF.

2. Selecting components of an MCF

2.1. Defining the approach: native, heterologous, or artificial

Designing an MCF begins with defining the product of interest. The desired product could be a native metabolite of the chassis organism (i.e., wild-type host), or additional metabolic capabilities may be required for a chosen chassis to produce the product of interest. It is important to note that simply the presence of a biosynthesis pathway does not guarantee that a particular chassis is the optimum choice. Even if a biosynthetic pathway is already present, often metabolic and/or enzyme engineering strategies may be required to increase metabolic flux through the pathway to arrive at yields needed for industrial production. In a recent example, an MCF was created using *Saccharomyces cerevisiae* along with a computationally-derived metabolic engineering strategy for succinate overproduction. Even though succinate is produced naturally by wild-type *S. cerevisiae*, it is consumed by the TCA cycle. An engineered strain of *S. cerevisiae* capable of producing industrially-relevant quantities of succinate (>40-fold yield improvement over wild-type) was created by deleting the succinate dehydrogenase (responsible for succinate depletion) and the 3-phosphoglycerate dehydrogenase isoenzymes. The resulting mutant up-regulated isocitrate conversion to succinate and glyoxylate to counteract serine and glycine deficiency [4]. Additional computationally-derived metabolic engineering strategies are discussed throughout.

Another common approach to creating an MCF is to install a heterologous or artificial de novo biosynthetic pathway in a chassis organism to arrive at a new product. The desired product could be (i) native to a microbe that is difficult to culture/engineer, (ii) from a higher organism (e.g., a plant) whose industrial production is not cost effective, or (iii) non-native to all microbes and a product of artificial metabolism. In addition, the MCF has also provided a convenient way of producing new derivatives of a compound of interest. As an example, phenylpropanoids, including resveratrol, are natural plant secondary metabolites that have demonstrated therapeutic benefits and commercial value. These and more bioavailable derivatives of resveratrol were sought from an MCF. A de novo biosynthetic pathway for the formation of resveratrol in *Escherichia coli* was constructed using heterologous enzymes from bacteria and plants [5], and it was later expanded by the addition of a glycosyltransferase (from *Bacillus*), which enabled synthetic production of resveratrol glucoside derivatives (i.e., resveratrol 3-O-glucoside and resveratrol 4′-O-glucoside) in an *E. coli* MCF [6]. The use of enzymes here in their natural function, with natural substrates toward the production of phenylpropanoids, is an example of a heterologous biosynthetic pathway. However, the use of the glycosyltransferase to produce new compounds relies on enzyme promiscuity (i.e., the ability of an enzyme to accept multiple substrates [7,8]). It is with promiscuous enzymes that novel arrangements of enzymes can give rise to artificial de novo biosynthetic pathways that allow MCFs to produce new chemicals. While there are many published accounts, some examples include the production of: (i) isobutanol [9,10], (ii) hydrocarbons [11], (iii) styrene [12], (iv) 3-hydroxybutyric acid [13], (v) native silk protein [14], and (vi) isoprenoids [15,16]. Most naturally occurring enzymes maintain a spectrum of substrate promiscuity to maximize evolutionary fitness and that promiscuity can be engineered [17]. Computational tools for enzyme engineering along with tools for artificial pathway synthesis and assembly are discussed below. However, first, the guidelines for selecting/engineering an optimal MCF chassis (i.e., host organism) are discussed.

2.2. Selecting the MCF Chassis

The choice of MCF chassis can vary greatly and is generally made according to: (i) the difficulty of metabolic engineering needed (and available toolsets), (ii) the nature and toxicity of the product, and (iii) the metabolic requirements (i.e., pathways, precursors, and cofactors) needed to produce the product. A list of common MCF chasses and their advantages/disadvantages is given in Table 1. While *E. coli* and yeast still dominate as popular chasses due to their well-developed genomic tools, this is expected to change. In the near-term, new genomic toolsets will allow the MCF chassis to take advantage of biodiversity, natural capabilities, and synergies. Ultimately, the minimal

Table 1
Common MCF chasses.

Organism[a]	Advantages/disadvantages of chassis	References
Clostridium sp.	Sporulating obligate anaerobes; gene knockout and over-expression tools available but can be very difficult to grow and engineer; ability to use a wide variety of complex substrates including lignocellulose and CO_2 to sustain growth	[3,20–22]
Corynebacterium glutamicum	A well-established industrial workhorse; genetic tools are available	[23]
Escherichia coli	Most well-characterized prokaryote; already used broadly in industry; genomic tools and systems biology datasets are widely available	[24]
Myxococcus xanthus	Effective host for myxobacterial, polyketide, and deltaproteobacterium synthesis pathways	[25]
Pseudomonas putida	Ease of cultivation and well established transformation techniques; capable of rapid growth, homologous recombination, and post-translational modifications; swappable genetic elements with *E. coli*	[26,27]
Saccharomyces cerevisiae	Well characterized and widely used in industry; genomic tools and systems biology datasets are widely available. Difficulties with anaerobic fermentation	[4]
Streptomyces sp.	Synthesis of polyketide derivatives	[1,28]
Chinese Hamster Ovary (CHO)	Production of sialylated and glycosylated proteins, recombinant human proteins, and high value pharmaceutical therapeutics; large production and cultivation costs	[29]
Taxus plant cells	Effective synthesis of toxic secondary plant metabolites; slow growing and low yields	[30]

[a] CHO and plant cells are included for comparison with traditional MCFs.