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Increasing carbon source uptake rates to improve chemical productivity in metabolic engineering

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Introduction

By transforming inexpensive raw materials into value-added chemicals via engineered microorganisms, metabolic engineering is emerging as a translational field with commercial impact. Broadly, metabolic engineering research typically involves facets of tool development, proof of concept, and production improvements for a target compound. It is this latter category that is fraught with practical constraints and limitations when considering possible commercialization. How do you produce enough of a desired compound for a commercially viable process when you have a physiologically limited input of starting materials and a continuous waste/drain of materials in the form of cells and undesired products? This challenge gets to the heart of microbial physiology where organism-specific nuances of transport mechanisms, catabolite repression, intracellular metabolite toxicity, basal metabolic burden, and regulation need to be considered [1•].

Much of metabolic engineering focuses on designing/implementing novel pathways and removing undesirable pathways that drain cellular resources. In this review, we will focus on strategies that improve carbon source uptake rates that represent the input of materials available for downstream bioconversion. The strategies include: first, use of fast-growing organisms with high carbon source uptake rates; second, evolutionary engineering of strains to increase growth rates and carbon source uptake rates; third, mutagenesis of transport proteins to increase carbon source uptake rates; fourth, decoupling of cell growth from chemical synthesis; and fifth, development of cell-free systems (Figure 1). We end with some thoughts on unifying the criteria that are used to assess chemical productivity and future prospects.

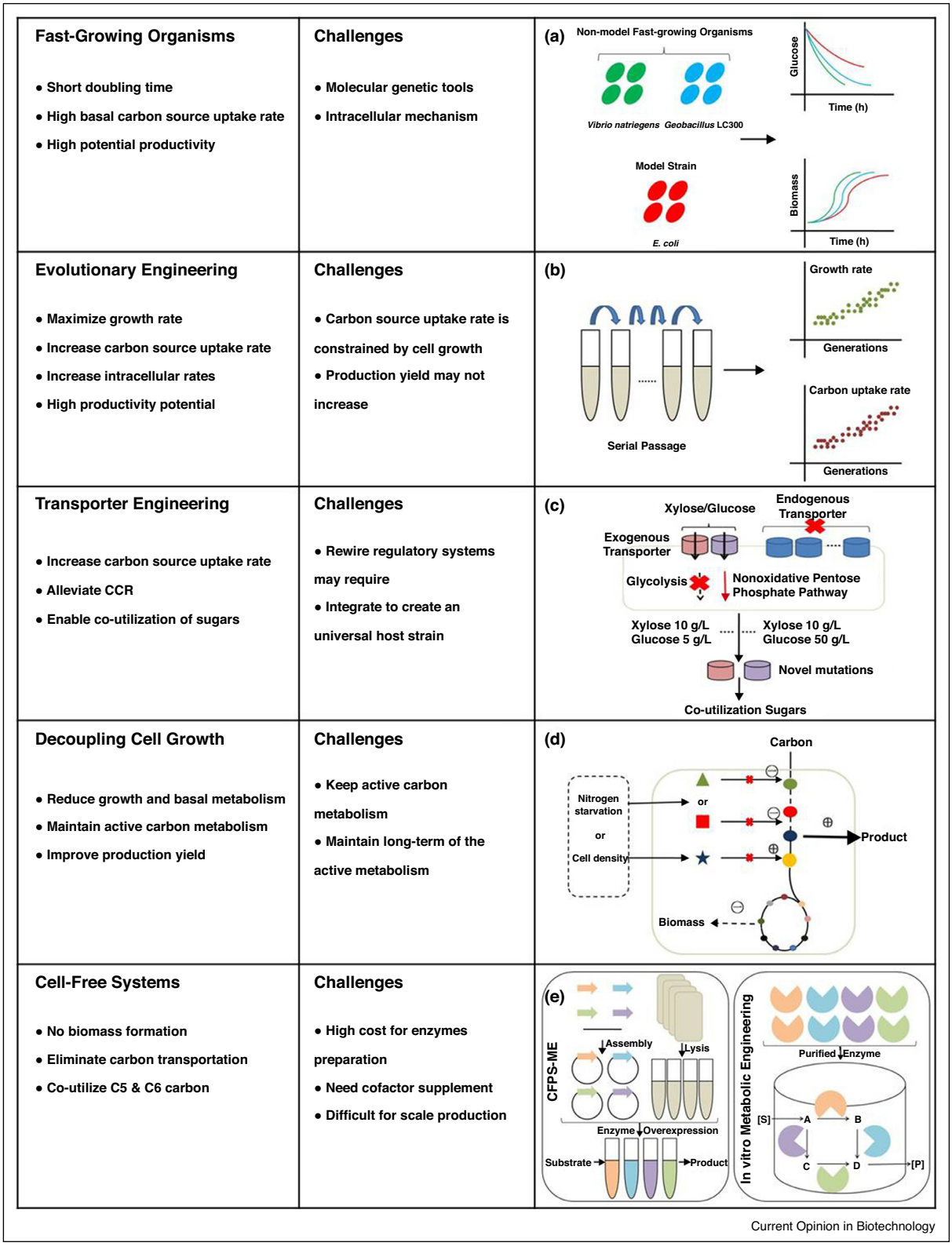
Fast-growing organisms

The majority of metabolic engineering research has been done with a small number of model organisms such as *Escherichia coli* and *Saccharomyces cerevisiae* to leverage existing knowledge and tools. As new organisms continue to be identified and studied, it will be increasingly valuable to evaluate their metabolic capabilities both in terms of effectiveness and efficiency for biochemical production. While bioprospecting for novel functions can be fruitful, it is also important to consider novel organisms as potential strain platforms. In particular, metabolic efficiency and substrate uptake can strongly influence biochemical productivity and titer.

One specific area that is often relegated to a secondary consideration is an organism's basal growth rate and substrate uptake rate. While a fast-growing organism may be detrimental to biochemical production (due to resources being channeled to biomass), growth rate is typically directly proportional to the substrate uptake rate. To illustrate this, when looking at several different organisms with different growth conditions, there is a linearly proportional relationship where faster growth rates are accompanied by faster carbon source uptake rates (in carbon-limited growth), shown in Figure 2. Thus, a fast-growing organism should have a proportionally faster basal uptake rate that would provide the potential for higher productivity (more mass per time available for conversion). In addition, fast-growing organisms with increased cell density would also increase biochemical titer if the supply of carbon source is not limiting biochemical synthesis.

Recently, an exceptionally fast-growing organism called *Vibrio natriegens* has been isolated. *V. natriegens* is reported to have a doubling time between 7 to 10 minutes [2,3] with correspondingly elevated uptake rates. The annotated genomic sequence is readily available for this organism [4,5], and, recently, a wide range of genetic tools have been developed and described to engineer *V. natriegens* [6•]. The fast growth of *V. natriegens* is potentially associated with a stream-lined genome that facilitates fast chromosomal replication and abundant protein synthesis machinery (e.g. high rRNA and ribosome levels). In

Figure 1



Overview of advantages and challenges of different strategies to increase carbon source uptake rates. **(a)** fast-growing organisms have short doubling time and high carbon source uptake rate; **(b)** laboratory adaptive evolution conducted by serial passage and carbon-limited conditions leads to increased growth rates and carbon source uptake rates; **(c)** mutagenesis approaches with evolutionary engineering generate key genetic