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Review

Systems metabolic engineering of microorganisms to achieve large-scale production of flavonoid scaffolds

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ABSTRACT

Flavonoids possess pharmaceutical potential due to their health-promoting activities. The complex structures of these products make extraction from plants difficult, and chemical synthesis is limited because of the use of many toxic solvents. Microbial production offers an alternate way to produce these compounds on an industrial scale in a more economical and environment-friendly manner. However, at present microbial production has been achieved only on a laboratory scale and improvements and scale-up of these processes remain challenging. Naringenin and pinocembrin, which are flavonoid scaffolds and precursors for most of the flavonoids, are the model molecules that are key to solving the current issues restricting industrial production of these chemicals. The emergence of systems metabolic engineering, which combines systems biology with synthetic biology and evolutionary engineering at the systems level, offers new perspectives on strain and process optimization. In this review, current challenges in large-scale fermentation processes involving flavonoid scaffolds and the strategies and tools of systems metabolic engineering used to overcome these challenges are summarized. This will offer insights into overcoming the limitations and challenges of large-scale microbial production of these important pharmaceutical compounds.

Contents

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1. Introduction

Plant flavonoids, encompassing more than 9000 substituted moieties, have recently attracted increasing research interest due to their diverse biological functions (Wang et al., 2011b). These biological functions, which include antiviral, antibacterial, anti-obesity, and anticancer activities, are useful for the treatment of several human pathologies (Si et al., 2010; Wang et al., 2009). For example, genistein and daidzein are highly effective at improving lipid metabolism and lowering blood sugar by enhancing lipid and glucose metabolism (Ae Park et al., 2006). Numerous studies have demonstrated the beneficial effects of naringenin in normalizing lipids in diabetes and inhibiting proliferation of hepatitis C virus (Goldwasser et al., 2010; Kim et al., 2003).

The core flavonoid structure consists of two benzene rings interconnected by a heterocyclic ring. Based on their molecular structures, flavonoids can be divided into six major categories: isoflavones, flavanones, flavones, flavonols, catechins, and anthocyanins (Fowler and Koffas, 2009). Among these, the flavonoid scaffolds, naringenin and pinocembrin, have significant pharmaceutical activities (Santos et al., 2011; Wu et al., 2013a). From these scaffolds, over 8000 different chemical structures can be generated through the actions of functionalizing enzymes (Fowler and Koffas, 2009). Hence, immense efforts have been dedicated to developing sustainable processes for the production of these products.

Currently, naringenin and pinocembrin are mainly extracted from plants. However, their limited availability and the complicated downstream purification of these compounds are major bottlenecks in this process (Wang et al., 2011b). The use of toxic chemicals and extreme reaction conditions also overshadows chemical synthesis routes (Santos et al., 2011). Alternatively, microbial synthesis is emerging as a promising approach due to its several advantages, including use of environmentally friendly materials, low energy requirement, and low waste emissions. Genetically tractable microorganisms including *Escherichia coli* (Wu et al., 2013a) and *Saccharomyces cerevisiae* (Koopman et al., 2012), engineered with heterologous metabolic pathways (Fig. 1), have afforded tremendous progress in producing these compounds.

To date, microbial production has only been achieved on the laboratory scale (Leonard et al., 2008; Wu et al., 2013a). Further development of these potent natural therapeutic agents is hampered by many obstacles including: (i) a heavy reliance on the addition of expensive phenylpropanoic precursors (Leonard et al., 2008) or aromatic amino acids (Miyahisa et al., 2005), (ii) the requirement of two different media for cell proliferation and flavonoid production (Santos et al., 2011; Wu et al., 2013a), and (iii) the low intracellular concentration of malonyl-CoA. The emergence of systems metabolic engineering, which combines systems biology with synthetic biology and evolutionary engineering at the systems level, offers new perspectives on strain and process optimization (Sagt, 2013). This review focuses on the general strategies and tools that are integral to systems metabolic engineering and examples of successful applications (Table 1), which offer insights into overcoming the limitations and challenges of large-scale microbial production of these important pharmaceutical compounds.

2. Construction of synthetic metabolic pathways

Naringenin and pinocembrin cannot be obtained through native metabolic pathways from renewable resources in most microorganisms (Santos et al., 2011; Wu et al., 2013a). Novel strategies and tools are required to establish synthetic pathways for the efficient formation of these phytochemicals. In many cases, enzymes and pathways available in nature are used as a diverse collection of genes for reconstructing a synthetic pathway (Lee et al., 2012). Furthermore, the considerable body of genetic, genomic, and enzymatic data can facilitate the design of more efficient metabolic pathways using enzymes derived from various organisms (Lee et al., 2012). Computational algorithms based on flux balance analysis (FBA) can be developed to predict genetic perturbation targets that would channel carbon flux toward target chemicals (Medema et al., 2012). Directed evolution and site-directed mutagenesis can also provide new catalytic functions (Lee et al., 2012; Wang et al., 2011a) (Fig. 2).

2.1. De novo pathway design

Designing optimal pathways for the production of flavonoids from inexpensive, safe, and renewable resources is the first step in reconstructing the appropriate synthetic metabolic pathways in microorganisms. The massive amounts of genomic information generated from high-throughput sequencing provide tremendous possibilities for designing synthetic metabolic pathways (Mitchell, 2011). The best candidate enzymes derived from various organisms are then heterologously or combinatorially introduced to construct a novel synthetic pathway (Santos et al., 2011).

Since 2003, researchers have been intensively investigating the microbial production of flavonoids (Hwang et al., 2003; Leonard et al., 2007, 2008; Miyahisa et al., 2005). Recently, more economical processes for *de novo* production of naringenin and pinocembrin without the need to feed precursors have been developed (Koopman et al., 2012; Santos et al., 2011; Wu et al., 2013a). These advances were achieved by introducing a heterologous synthetic pathway, consisting of phenylalanine/tyrosine ammonia lyase (PAL/TAL), 4-coumarate:CoA ligase (4CL), chalcone synthase (CHS), and chalcone isomerase (CHI), into *E. coli* strains (Santos et al., 2011; Wu et al., 2013a) or *S. cerevisiae* (Koopman et al., 2012). The selection of appropriate genetic sources such as TAL/PAL from *Rhodotorula glutinis* contributed significantly to the *de novo* synthesis of naringenin and pinocembrin. Such strains could produce 29 mg/L naringenin (Santos et al., 2011) and 40 mg/L pinocembrin from glucose in *E. coli* (Wu et al., 2013a) or 109 mg/L naringenin from glucose in *S. cerevisiae* (Koopman et al., 2012).

2.2. Genome-scale metabolic network modeling

Synthesis of desired products is often achieved *via* more than one metabolic pathway (Xu et al., 2013c). In addition to designing optimized heterologous pathways, it is essential to effectively redirect endogenous carbon flux toward target products.

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