Journal of Biotechnology 163 (2013) 166–178

Contents lists available at SciVerse ScienceDirect

Journal of Biotechnology

journal homepage: www.elsevier.com/locate/jbiotec

Metabolic engineering of microorganisms for the synthesis of plant natural products

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ARTICLE INFO

Article history:
Received 30 March 2012
Received in revised form 29 May 2012
Accepted 1 June 2012
Available online 9 June 2012

Keywords:
Secondary metabolism
Plant natural products
Synthetic biology
Metabolic engineering
Combinatorial biosynthesis
Value-added products
Flavonoids
Stilbenes
Isoprenoids
Terpenoids
Alkaloids
Phenylpropanoids
Lignans
Coumarins

ABSTRACT

Of more than 200,000 plant natural products known to date, many demonstrate important pharmacological activities or are of biotechnological significance. However, isolation from natural sources is usually limited by low abundance and environmental, seasonal as well as regional variation, whereas total chemical synthesis is typically commercially unfeasible considering the complex structures of most plant natural products. With advances in DNA sequencing and recombinant DNA technology many of the biosynthetic pathways responsible for the production of these valuable compounds have been elucidated, offering the opportunity of a functional integration of biosynthetic pathways in suitable microorganisms. This approach offers promise to provide sufficient quantities of the desired plant natural products from inexpensive renewable resources. This review covers recent advancements in the metabolic engineering of microorganisms for the production of plant natural products such as isoprenoids, phenylpropanoids and alkaloids, and highlights general approaches and strategies to gain access to the rich biochemical diversity of plants by employing the biosynthetic power of microorganisms.

1. Introduction

Plant secondary metabolites are not directly involved in growth, development or reproduction of plants, but have important functions in plant defense and signaling, or serve as pigments or fragrances. Unlike primary metabolites, which are mostly the same across the broad spectrum of all living organisms, plant secondary metabolites can vary from species to species and comprise a diverse array of complex chemical structures.

Since time immemorial, plant natural products (PNPs) such as isoprenoids, alkaloids and flavonoids, which are all plant secondary metabolites have been used by humans as colorants, flavors and fragrances. Due to the scarce availability and high price of natural products, synthetic compounds have gained more and more importance in the food industry in the last decades. However, nowadays customers demand natural products as a consequence of proven toxicological effects of some synthetic compounds (Sowbhagya and Chitra, 2010). Usually, PNPs have no nutritional value, but a diet rich in PNPs can boost the immune system (Licciardi and Underwood, 2011) or protect the human body from free radicals and are thus believed to prevent or suppress carcinogenesis (Guo et al., 2009; Tan et al., 2011). Ever since, PNPs were also of great importance as pharmaceutical drugs. In the last decades, PNPs were heavily exploited in drug discovery by high-throughput screening (HTS) of natural product libraries (Koehn and Carter, 2005) and almost half of all pharmaceuticals brought to the market from 1981 to 2006 were (plant) natural products or directly derived thereof (usually by semisynthetic modifications) (Newman and Cragg, 2007). This trend continues as more recent reports show, that 19 new (plant) natural product-based pharmaceutical drugs were approved for marketing worldwide in the last five years (Mishra and Tiwari, 2011). These numbers are noteworthy, especially when considering that pharmaceutical companies nowadays favor robotic HTS of vast libraries of synthetic molecules, which are generated by combinatorial chemistry (Newman, 2008). However, due to the limited chemical diversity and structural complexity of such synthetic libraries, as well as great success of natural product-derived

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http://dx.doi.org/10.1016/j.jbiotec.2012.06.001

drugs on the market in the last years, screening of untapped biological resources for new natural products is expected to be continued in the future (Li and Vederas, 2009).

Once a potent PNP is discovered, the ability to produce enough material for industrial or clinical applications becomes the main limiting factor. In the last years significant work has been made on improving PNP yields in native plant sources (Wu and Chappell, 2008) and also combinatorial biosynthesis in plants has made great leaps forward (Pollier et al., 2011). Despite all progress, final extraction from native plant sources is often problematic since PNPs tend to accumulate at low quantities over long growth periods. Furthermore, purification of the desired products requires separation from a multitude of often structurally very similar compounds and overall yields are also subject to environmental and regional factors (Chemler and Koffas, 2008). Recently, plant cell cultures of innately undifferentiated cambial meristematic cells from *Taxus cuspidata* proved to be promising for the production of several PNPs (Lee et al., 2010). Nevertheless, application of plant cell cultures is limited due to inconsistent performance and economic constraints associated with slow growth and low product yield (Kolewe et al., 2008). Total chemical synthesis or semisynthesis from isolated precursors poses many challenges for chemists and has little practical value for the production of complex PNPs with multiple chiral centers and labile connectivities (Pickens et al., 2011). Indeed, elegant methodologies for the synthesis of complex PNPs exist, but with an increasing number of separate steps, synthetic routes become impractical as the overall yield decreases, whereas the amount of resources consumed and (toxic) waste accumulated increase (Newhouse et al., 2009).

Microbial production of PNPs is a promising alternative with several advantages in comparison to above-mentioned approaches. Since many decades microorganisms have been increasingly used to produce a broad range of value-added compounds with numerous applications in the food, chemical and pharmaceutical industries. Important examples of these compounds include many (proteinogenic) amino acids, organic acids, vitamins, fine-chemicals, biofuels as well as a large number of pharmaceutical drugs (Becker and Wittmann, 2011; Du et al., 2011; Wendisch et al., 2006). Microbial production has the advantage of being much more environmentally friendly compared to chemical synthesis, since it avoids the use of organic solvents, heavy metals and strong acids or bases. Furthermore, in contrast to natural product synthesis in plants and plant cell cultures, microbial production based on renewable feed stocks is inexpensive and the rapid growth of microorganisms allows short production times. Unlike traditional synthetic chemistry-based routes, microbial fermentations are readily scalable from the lab bench to industrial-sized fermenters of several hundred cubic meters. In addition, the genetic accessibility of industrially relevant microorganisms such as *Escherichia coli*, *Corynebacterium glutamicum*, *Bacillus subtilis*, *Pseudomonas putida* or *Saccharomyces cerevisiae* allows construction of tailor-made recombinant strains by metabolic engineering to modify metabolic profiles according to individual production purposes. This includes also the heterologous expression of whole metabolic pathways of plants in microorganisms to confer the capability for PNP synthesis. Since recombinant microorganisms are usually void of competing pathways to the heterologously expressed pathway from plants, desired PNPs are typically made in the cell as chemically distinct substances (Chemler and Koffas, 2008). This in turn simplifies downstream processing in comparison to product purification from plants or plant cell cultures.

This review summarizes the recent advances in metabolic engineering of microorganisms for the synthesis of PNPs with a focus on isoprenoids, phenylpropanoids and alkaloids as well as the metabolic engineering strategies leading to PNP-producing microorganisms. Although many metabolic engineering approaches are PNP-specific, we highlight general challenges and technological strategies for microbial PNP synthesis and give an outlook on potential future developments of this research field.

2. Plant natural product synthesis in microorganisms

Considering that more than 200,000 PNPs are known (Osbourn and Lanzotti, 2009), it is astonishing that PNPs are derived from only a handful of primary metabolites formed in few central metabolic pathways (Fig. 1). Most of these chemically diverse compounds fall into three classes of natural products: firstly, isoprenoids including sterols and carotenoids; secondly, phenylpropanoids and phenylpropanoid-derived compounds such as flavonoids, stilbenes and lignans; and thirdly alkaloids as nitrogen containing PNPs. Other PNPs such as polyketides, sulfur- and nitrogen containing glucosinolates, or polyacetylenes as fatty acid-derived natural products are not covered by this review. Readers, who are interested these PNPs are referred to other recent reviews and research articles (Gao et al., 2010; Minto and Blacklock, 2008; Moldrup et al., 2011; Niraula et al., 2010; Sonderby et al., 2010).

2.1. Isoprenoids

With more than 40,000 known structures, including sterols and carotenoids, isoprenoids (also known as terpenoids), represent the largest class of PNPs (Bohlmann and Keeling, 2008). Many carotenoids and monoterpenes such as menthol, pinene or limonene for the cosmetic-, fragrance- and food industry are synthesized by plants in larger quantities and thus can be produced and purified rather simply from plants (Namitha and Negi, 2010; Schewe et al., 2009). However, many isoprenoids with significant pharmaceutical importance, most notable examples being the antimalarial drug artemisinin and the anticancer drug paclitaxel (Fig. 2), have a low abundance in plants. All isoprenoids originate from the five-carbon building blocks isopentenyl-pyrophosphate (IPP) and its isomer dimethylallyl-pyrophosphate (DMPP), both derived from either the mevalonate (MEV) pathway, employed in many prokaryotes and in the cytosol of higher plants, or the 2C-methyl-D-erythritol-4-phosphate (MEP) pathway, which is used in most prokaryotes, all eukaryotes and the chloroplasts of higher plants (Fig. 1) (Kuzuyama and Seto, 2003; Rohmer et al., 1993). The nomenclature for the latter pathway was confusing as several other names, such as DXP-, DOXP-, Rohmer-, or non-mevalonate pathway have been used in literature. Only recently, the pathway was officially named the "MEP pathway", according to the first committed precursor in the biosynthetic route of *E. coli* (Phillips et al., 2008). Condensation of the two C_5-pyrophosphates and larger IPP- and DMAPP derived building blocks such as farnesyl pyrophosphate (FPP, C_{10}), geranyl pyrophosphate (GPP, C_{20}) and geranylgeranyl pyrophosphate (GGPP, C_{40}) give access to monoterpenes (C_{10}, e.g. menthol), sesquiterpenes (C_{15}, e.g. artemisinin), diterpenes (C_{20}, e.g. paclitaxel), triterpens (C_{30}, e.g. steroids), and tetraterpenes (C_{40}, carotenoids) (Fig. 2). All organisms are able to synthesize isoprenoid structures and hence can provide IPP, DMAPP and other longer isoprenoid backbones as precursors (Hunter, 2007). However, only a few microorganisms such as *E. coli* and *S. cerevisiae* have been engineered for production of plant isoprenoids. These microbes share the advantages of having been studied over decades, and thus their physiology, biochemistry, genome, transcriptome, proteome and metabolome is already well understood (Tyo et al., 2007). This knowledge as well as long lasting experience in industrial production of other small metabolites and proteins with both microorganisms make *E. coli* and *S. cerevisiae* excellent hosts or "platform organisms" for the production of isoprenoids as well as other (plant) natural products.