Thieme

Ethnobotany and Medicinal Plant Biotechnology: From Tradition to Modern Aspects of Drug Development*

Author
Oliver Kayser

Affiliation
TU Dortmund University, Biochemical and Chemical Engineering, Technical Biochemistry, Dortmund, Germany

Key words
ethnobotany, pharmacognosy, phytochemistry, systems biology, metabolic engineering, bioengineering, synthetic biology, microbial cell factories

received February 28, 2018
revised May 9, 2018
accepted May 10, 2018

Bibliography
DOI https://doi.org/10.1055/a-0631-3876
Published online May 24, 2018 | Planta Med 2018; 84: 834–838 © Georg Thieme Verlag KG Stuttgart · New York | ISSN 0032-0943

Correspondence
Prof. Dr. Dr. Oliver Kayser
TU Dortmund University, Biochemical and Chemical Engineering
Emil-Figge-Straße 66, Dortmund, Germany
Phone: + 49 2 31 75 57 48, Fax: + 49 23 17 55 73 82
oliver.kayser@tu-dortmund.de

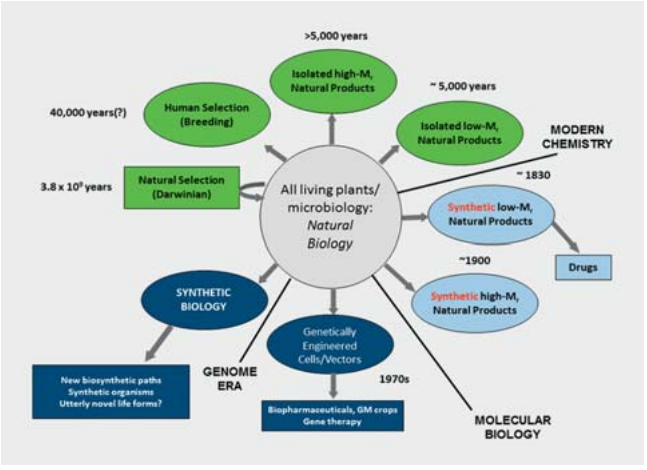
ABSTRACT

Secondary natural products from plants are important drug leads for the development of new drug candidates for rational clinical therapy and exhibit a variety of biological activities in experimental pharmacology and serve as structural template in medicinal chemistry. The exploration of plants and discovery of natural compounds based on ethnopharmacology in combination with high sophisticated analytics is still today an important drug discovery to characterize and validate potential leads. Due to structural complexity, low abundance in biological material, and high costs in chemical synthesis, alternative ways in production like plant cell cultures, heterologous biosynthesis, and synthetic biotechnology are applied. The basis for any biotechnological process is deep knowledge in genetic regulation of pathways and protein expression with regard to todays "omics" technologies. The high number genetic techniques allowed the implementation of combinatorial biosynthesis and wide genome sequencing. Consequently, genetics allowed functional expression of biosynthetic cascades from plants and to reconstitute low-performing pathways in more productive heterologous microorganisms. Thus, *de novo* biosynthesis in heterologous hosts requires fundamental understanding of pathway reconstruction and multitude of genes in a foreign organism. Here, actual concepts and strategies are discussed for pathway reconstruction and genome sequencing techniques cloning tools to bridge the gap between ethnopharmaceutical drug discovery to industrial biotechnology.

Ethnopharmacy

For centuries, the history of pharmacy and drug discovery has been identical with the history of pharmacognosy and ethnobotany. Based on the story of curare, quinine, and later morphine or artemisinin, plenty of natural products and plant extracts have been used in ethnopharmacology [1]. According to Heinrich et al., ethnopharmacology by its definition draws its attention to the scientific study of indigenous drugs but does not explicitly address the issue of searching or even cultivating and breeding for an enhanced yield. As stated by the World Health Organization (WHO) [2], medicinal plants are an important element of indigenous medical systems in many parts of the world. About 80% of the world's population relies on herbal medicinal products due to limited access to Western medicine with regulated mostly synthetic or biotechnological drugs [3]. However, not only so-called low-income countries but also Europe have benefited from the close sharing of knowledge with other mostly tropical countries. Many of today's modern drugs like local anesthesia (e.g., cocaine), pain killer (e.g., morphine), and antineoplastic drugs (e.g., podophyllotoxin, camptothecin) have their structural skeleton in a natural product analogue. According to Newman and Cragg, about 40% of all clinically used drugs originate from natural products

* Dedicated to Professor Dr. Robert Verpoorte in recognition of his outstanding contribution to natural products research.



▶ **Fig. 1** From breeding to synthetic biology, biological systems toward natural product biotechnology.

[1,4,5]. The role of traditional medicine is to provide medicinal active plants in the drug discovery process [6–8].

Even though our ancestors did not have any detailed knowledge about chemical structures, natural products formed the basis of many medicines. The reasons for this are manifold, but it is likely that the ability of nature to create fantastic complex and structurally diverse molecules is the most convincing argument that natural products show very broad biological activity. Besides of the history of natural products based on plants, we should not forget that microorganisms are the real chemists that provide an unbelievably high number of secondary natural products [9]. About 250,000 higher plants are known and about 120,000 isolated and structure elucidated compounds have been published. A closer look at the biodiversity of microorganisms shows that we even do not know how many of them exist on this planet. Some estimates suggest that we know roughly only about 1% of living microorganisms and we have just started to understand the chemical diversity of a few thousand molecules.

In its primitive form, plant breeding started after the invention of agriculture. Breeding of crops as wild strains from nature in prehistoric times was also the starting point of ancient plant breeders. Depending on classical tools to develop new and improved strains for food, they focused mainly on crop plants like maize and corn [10,11]. As depicted in ▶ **Fig. 1**, plant breeders simply selected food plants with desirable characteristics and applied crossing techniques. Later, Gregor Mendel's experiments on hybridization [12] had a strong impact, but with the advent of biotechnology, breeders were now in the position to incorporate molecular tools in their breeding work. Optimization of medicinal plants and natural product yield by breeding played no major role and started with the 20th century. New opportunities in gene technology paved the road to metabolic engineering, and in continuation with metabolomics tools [13], synthetic biology is the most recent milestone in this development. Integration of life science disciplines will have a new potential we cannot fully oversee today. Heterologous assembly of artificial pathways, crossing species borders, and redesigning cellular metabolism are only some examples and will be briefly introduced [14]. Today, secondary natural products like morphine [15], vanillin [16], resveratrol [17], and tetrahydrocannabinolic acid [18] are already biosynthesized in microorganisms.

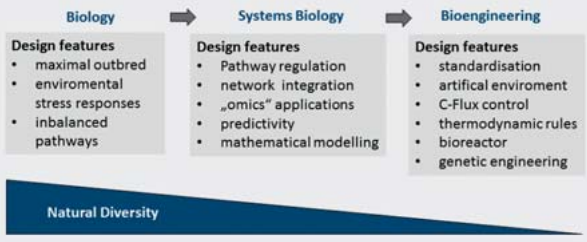
In various online databanks information on the genome, transcriptome, and metabolome is accessible. For instance, transcriptome data of 1000 (medicinal) plants from the 1KP project are ready to use [19,20]. In the times of "omics" with floods of genome and transcriptome, data are coming over us and scientists do not fully understand how to dig for new drug leads and how to use all data for a smart and lean drug discovery process [21]. In principal, biologists, and physicians have accumulated an enormous amount of digital information and application of omics tools belongs to standard operation methods in the context of quality control and identification of natural products [22,23]. Since the finalization of the human genome project and other related projects to explore human proteome, transcriptome, or epigenome, the pharma industry has not released one new drug entity that has kept the promise of the uprising genome era. On the contrary, new drug entities still come from classical drug discovery processes including natural products [24]. The main reason for this is that creative nature still provides extremely complex chemical skeletons that chemists cannot imagine in their labs. As chemists, pharmacists, and physicians, we find molecules fascinating with overbridging ring systems and stereochemistry as known for paclitaxel, quinine, or artemisinin.

We acknowledge the beauty of these molecules, but we mostly do not see the enormous problems we face when we try to get these compounds as clinical drugs in their final dosage form to the patient. Natural products are of high interest because of high price (▶ **Table 1**); they are considered "orphan drugs" by pharmaceutical industry. Mostly they cannot be obtained in a sufficient amount from plant source; even emerging outdoor cultivation (e.g., scopolamine), production in plant cell cultures (e.g., shikonin, paclitaxel), or collection from the wild (e.g., podophyllotoxine) are making big challenges why the pharma industry is reluctant to develop pure natural products as drug candidates. Other alternatives to produce are in general costly and hard to translate into the pharmaceutical industry. As an example, outdoor production of *Duboisia myoporoides* R.Br. (Solanaceae) to extract scopolamine is costly and demands high logistic effort [25]. Plant cell callus cultures do not provide high yields and deliver yields in low range of mg/L (taxanes: 5–10 mg/L; hyoscyamine: 5 mg/L; gingengosides: 28–145 mg/L) [26]. With the exception of paclitaxel [27], no drug was ever produced in a commercial-scale in plant suspension cultures. In the last 40 years, shikonin as cosmetic has been developed and entered for a short time the market only [28].

Impact of metabolic engineering and synthetic biology changed the rules of the game and microorganisms became interesting for implementation of full plant pathways expression. Starting from genome sequencing of medicinal plants to describe and formally explain and document physiological conditions to systems biology and bioengineering, chemical and genetic diversity was reduced to create a minimal cell as a high-performer platform organism (▶ **Fig. 2**). Applying engineering principles like mathematical modelling, statistical calculations for C-flux optimi-

▶ **Table 1** Plant-derived natural products of importance for the pharmaceutical industry.

Compound	Plant species	Need (to/y)	Price USD$/kg	Cultivation in ha	Reference
Artemisinin	*A. annua*	50–60	100	29,000	[42, 45]
Paclitaxel	*T. brevifolia*	0.5	26,000–38,000	Wild collection (750,000 yew trees/year)	[57]
Docetaxol	*T. brevifolia*	0.3	8200–43,200	Semisynthesis	[57]
Resveratrol	*V. vinifera*	10,000	600	Fermentation, synthesis	[58]
Ajmalicine	*R. serpentina*	0.3	1500	Not known	[59]
Anthocyanins	*V. vinifera*	2.0	2000	Not known	[60, 61]
Vincristine	*V. minor*	0.8	350,000	Not known	[57, 60, 61]
Colchicine	*C. autumnale*	5.0	6000	Not known	[60, 61]



▶ **Fig. 2** Reducing chemical and genetic diversity toward directed platform organisms for the production of natural products.

zation, and optimization of energy conversion (ATP, NAD[P]H) [29] were applied as we know by definition for the discipline of bioengineering (▶ **Fig. 2**).

Two natural products have heavily impacted pharmaceutical research and bioengineering in the last 20 years: paclitaxel and artemisinin, which were both game-changers in medicinal plant biotechnology and synthetic biology. Because of the economic importance and impact on bioengineering, both are outlined here and the development from first discovery to the final industrial production explained.

Bioengineering of Paclitaxel Production

In 1962, first samples of the Pacific yew tree (*Taxus brevifolia* Nutt., Taxaceae) were collected and tested by the National Cancer Institute for antineoplastic activity. After a lengthy development process, clinical trials started in 1984 and market authorization followed in 1994 [30, 31]. Besides of difficulties in structure elucidation and determination of the pharmacological profile, a main obstacle was supply of the pure active ingredient. First, extraction of bark material from the at least 50-year-old trees from the wild was most appropriate and problematic at the same time. *T. brevifolia* is a rather slow-growing tree producing paclitaxel in very low amounts (10 µg/kg) [30]. To treat all women in the United States suffering from ovarian cancer within a year, the whole population of the tree has to be cut down in the United States, which would lead to its full extinction. Meeting the annual therapeutic requirements of patients with ovarian cancer requires 15–20 kg of the drug. Extending the indication to other cancer types, the demand will increase to up to 250–300 kg/year, an amount that would need to be isolated from 145,000 tons of bark. Harvesting such an amount is unrealistic and unsustainable. The strategy for obtaining pure active ingredients shifted from plant extraction (1975–1990) to the commercial silvicultural production of baccatin II as biosynthetic precursor from *Taxus baccata* L., Taxaceae (1990–2002) to current (2003) *in vitro* fermentation technology [32–34]. The first study to develop and optimize a cell-based bioprocess started at the beginning of the 1990s and demonstrated that calli of *Taxus* sp. were able to produce paclitaxel and its precursors at least as efficiently as the plant [32]. Successful strategies to enhance yield of paclitaxel derived later from bioengineering, improved media composition, high producer cell lines, elicitors, phytohormones, and bioreactor design [35, 36]. At the beginning of 2010s, omics technologies pushed plant cell fermentation to rational approaches [37]. The primary task was to identify biosynthetic genes, enzymes and regulating factors, and systems biotechnology information. In the postgenomic age, high-throughput methods like transcription profiling, microarrays, modern PCR techniques, and proteomics will accelerate and catalyze discovery of new genes and proteins for creating either new biosynthetic pathways or to improve the catalytic rate of enzymes to overcome bottlenecks in the carbon flux. Since 2000, several studies have taken an empirical approach to the elucidation of gene regulatory mechanisms related to paclitaxel biosynthesis in different *Taxus* species. As an example, increase of geranyl diphosphate as central precursor for formation of taxadiene as first committed metabolite toward taxanes and upregulation of the limiting enzyme taxadiene synthase was groundbreaking finding from postgenomics. Later on, more biosynthetic steps were analyzed and enzymes either upregulated, modified by protein engineering, or fully substituted to increase production yield of paclitaxel 40-fold in fermentation cultures [38, 39].

Bioengineering of Artemisinin Production

The second model case of interest is artemisinin. This molecule is by structure, clinical indication, and social importance one of its kind. In 2016, Youyou Tu was awarded for her discoveries concerning a novel therapy against malaria, but also for discovery and

structure elucidation of artemisinin from *Artemisia annua* L., Asteraceae [40]. This plant, whose Chinese name is *qinghao*, was described vividly in the fourth century the book *Zhouhou Beiji Fang* (*The Handbook of Prescriptions for Emergencies*) by the Chinese physician Ge Hong as a treatment for malarial fever [41]. Drugs based on artemisinin have led to the survival and improved health of millions of people. But the story is much more exciting. Artemisinin can be considered as the first secondary natural product paving the road for plant based molecules toward metabolic engineering and synthetic biology. Artemisinin is a sequiterpene lactone with an overbridged trioxan ring system containing a peroxide. Besides this fact, artemisinin has seven stereocenters. For this reason, organic synthesis is expensive and not affordable for companies. As outlined for paclitaxel extraction, the yield of artemisinin of 0.4% is also very low in *A. annua*. In combined efforts by the Bill and Melinda Gates Foundation and the University of York, high-yield varieties (1.2–1.6%) have been produced by molecular breeding technologies. Nevertheless, the price of pure artemisinin has not decreased and at 150–1500 USD/kg remains still quite high [42]. Artemisinin and related combination therapies have become indispensable for most patients suffering from malaria because of resistance of *Plasmodium* parasites–transmitted by *Anopheles* flies–against classical quinine based drugs. In 2003, the University of California and the same above-mentioned foundation started a synthetic biology project to produce artemisinin in baker's yeast at lower cost. This ambitious challenge was supported by 40 million USD and led to the heterologous production of dihydroartemisinic acid [43]. Keasling et al. from the University of California identified all relevant genes and enzymes for the mevalonate pathway and early biosynthesis of sesquiterpene lactones by genome sequencing and high throughput gene design. A critical element of Keasling's work was the development of genetic tools to aid in the manipulation of microbial metabolism, particularly for low-value products that require high yields from sugar [44]. His laboratory developed single-copy plasmids for the expression of complex metabolic pathways. He constructed promoter systems that allow regulated control of transcription consistently in all cells of a culture and mRNA stabilization technologies to regulate the stability of mRNA segments. Furthermore, new protein engineering approach to attach several enzymes of a metabolic pathway onto a synthetic protein scaffold to increase pathway flux were also invented and applied [43]. The mevalonate pathway in baker's yeast was fully reconstructed, metabolic bottle necks identified, and carbon flux starting from glucose as substrate optimized to boost up farnesyl pyrophosphate as the main precursor for the first committed step to form amorpha-4,11-diene. Most of the time and efforts were spent on the elucidation of the early biosynthesis. In short, oxidation of the methyl group in position C12 by three cytochromes to artemisinic acid was a challenging task. All attempts to identify enzymes catalyzing artemisinic acid to artemisinin failed, and up to now, no enzyme has ever been detected. The question remains whether final synthesis is enzyme-based or simple photooxidation in the oil container of plant trichomes. After negotiations with the WHO, Novartis, and Sanofi, the heterologous biosynthesis of artemisinic acid was transferred to an industrial level [45]. In a semi-biotechnological step, a photochemical reaction step was coupled to produce artemisinin in a classical chemical engineering environment. In 2013, Sanofi announced the launch of a production facility in Garessio, Italy, to manufacture the antiplasmodial drug on a large scale [46]. Sanofi produced 25 tons of artemisinin in 2013, ramping up the production to 55–60 tons in 2014 and supplying approximately one-third of the global annual need for artemisinin. The price per kg is 350–400 USD/kg, roughly the same as the botanical source.

▶ **Table 2** Recombinant secondary natural plant products from heterologous production.

Compound	Production organism	Titer	Reference
Resveratrol	*E. coli*	1.4 g/L	[58]
	S. cerevisiae	5 g/L	[64]
Vanillin	*S. cerevisiae*	45 mg/L	[16, 62]
Naringenin	*S. cerevisiae*	474 mg/L	[63]
Dihydroartesimic acid	*S. cerevisiae*	100 mg/L	[43]
Artemsinic acid		25 g/L	[65]
Morphine	*S. cerevisiae*	131 mg/mL	[66]
Ginsensosides	*S. cerevisiae*	54–1189 mg/L	[52]

Future Bioengineering of Cell Factories for Natural Products

What can we expect in the future from synthetic biology and bioengineering? Plant secondary metabolites exhibit a variety of biological activities and therefore serve as valuable therapeutics. As outlined, the small amounts isolated from plants still do not meet market demands. The term "metabolic engineering" was introduced 25 years ago to describe the application of recombinant DNA technology for improving metabolic processes in organisms [47]. Nowadays, the idea of genetic modification has fully changed to the employment of alternative routes with the same catalytic function but better performance, even from microorganisms that have never produced any plant metabolite [14, 48]. Based on next-generation sequencing data, pathways are not just reconstructed from plants but are composed of genes from various species in order to maximize efficiency. Metabolic engineering in plants involves the modification of endogenous pathways to enhance production of the compound of interest (▶ **Table 2**) to minimize biosynthesis of unwanted side products and to increase the biosynthetic rate by smart pathway design [14, 49]. It does not matter if genetic principles are applied either for plants or heterologous production platforms (e.g., *Escherichia coli, Saccharomyces cerevisiae*) [48]. Most strategies fit in a universal way: (i) *in silico* engineering (e.g., CellDesigner, e-cell [50, 51]) of single steps in a pathway to understand metabolic flux of metabolites from the primary to the secondary pathway; (ii) balancing the energetic flow of ATP, NADPH, and NADH; (iii) blocking competitive pathways; (iv) introducing pathway short cuts that divert meta-

bolic flux in a particular way [52]; and (v) identifying network topologies and modifying signaling cascades [14, 53].

However, all mentioned approaches have only limited value as single biochemical events. The power of metabolic engineering will become clear if multiple steps are affected in the same pathway. That requires a deep understanding of the biochemical and genetic underlying principles and mostly highly sophisticated computing and bioinformatics [54, 55]. The potential of bioengineering to improve secondary natural product biosynthesis in a rational directed way by modulating individual steps has been demonstrated in the past, and we can expect in the near future revolutionary concepts [56]. It will be no surprise to work with artificial or minimal cells that are *in silico* designed from scratch and we will choose our pathway of interest by copy and paste on an office computer.

Conflict of Interest

The author declares no conflict of interest.

References

[1] Gurib-Fakim A. Medicinal plants: Traditions of yesterday and drugs of tomorrow. Mol Aspects Med 2006; 27: 1–93

[2] Heinrich M, Heneka B, Rimpler H, Ankli A, Sticher O, Kostiza T. Spasmolytic and antidiarrhoeal properties of the Yucatec Mayan medicinal plant *Casimiroa tetrameria*. J Pharm Pharmacol 2005; 57: 1081–1085

[3] World Health Organization (WHO). WHO Traditional Medicine Strategy: 2014–2023. Geneva, Switzerland: WHO Press; 2013

[4] Newman DJ, Cragg GM. Natural products as sources of new drugs from 1981 to 2014. J Nat Prod 2016; 79: 629–661

[5] Cragg GM, Newman DJ. Natural products: a continuing source of novel drug leads. Biochim Biophys Acta 2013; 1830: 3670–3695

[6] Harvey AL. Natural products in drug discovery. Drug Discov Today 2008; 13: 894–901

[7] Shen B. A new golden age of natural products drug discovery. Cell 2015; 163: 1297–1300

[8] Li JWH, Vederas JC. Drug discovery and natural products: end of an era or an endless frontier? Science 2009; 325: 161–165

[9] Clardy J, Fischbach MA, Walsh CT. New antibiotics from bacterial natural products. Nat Biotechnol 2006; 24: 1541–1550

[10] Fedoroff NV. Prehistoric GM corn. Science 2003; 302: 1158–1159

[11] Acquaah G. Principles of Plant Genetics and Breeding. Malden, MA: Blackwell Publishing; 2007: 3–15

[12] Mendel G. Versuche über Pflanzen-Hybriden. Sch Publ 1866; 1865: 3–47

[13] Heinrich M. Ethnopharmacy and natural product research – multidisciplinary opportunities for research in the metabolomic age. Phytochem Lett 2008; 1: 1–5

[14] Nielsen J, Keasling JD. Engineering cellular metabolism. Cell 2016; 164: 1185–1197

[15] Hawkins KM, Smolke CD. Production of benzylisoquinoline alkaloids in *Saccharomyces cerevisiae*. Nat Chem Biol 2008; 4: 564–573

[16] Hansen EH, Møller BL, Kock GR, Bünner CM, Kristensen C, Jensen OR, Okkels FT, Olsen CE, Motawia MS, Hansen J. *De novo* biosynthesis of Vanillin in fission yeast (*Schizosaccharomyces pombe*) and baker's yeast (*Saccharomyces cerevisiae*). Appl Environ Microbiol 2009; 75: 2765–2774

[17] Wolfson W. Evolva breeds small molecule drugs au naturel. Chem Biol 2009; 16: 577–578

[18] Degenhardt F, Stehle F, Kayser O. The Biosynthesis of Cannabinoids. In: Preedy VR, ed. Handbook of Cannabis and related Pathologies. Cambridge, MA: Academic Press; 2017: 13–23

[19] Matasci N, Hung LH, Yan Z, Carpenter EJ, Wickett NJ, Mirarab S, Nguyen N, Warnow T, Ayyampalayam S, Barker M, Burleigh JG, Gitzendanner MA, Wafula E, Der JP, dePamphilis CW, Roure B, Philippe H, Ruhfel BR, Miles NW, Graham SW, Mathews S, Surek B, Melkonian M, Soltis DE, Soltis PS, Rothfels C, Pokorny L, Shaw JA, DeGironimo L, Stevenson DW, Villarreal JC, Chen T, Kutchan TM, Rolf M, Baucom RS, Deyholos MK, Samudrala R, Tian Z, Wu X, Sun X, Zhang Y, Wang J, Leebens-Mack J, Wong GKS. Data access for the 1, 000 Plants (1KP) project. Gigascience 2014; 3: 17

[20] Chen S, Xiang L, Guo X, Li Q. An introduction to the medicinal plant genome project. Front Med 2011; 5: 178–184

[21] Ulrich-Merzenich G, Panek D, Zeitler H, Wagner H, Vetter H. New perspectives for synergy research with the 'OMIC'-technologies. Phytomedicine 2009; 16: 495–508

[22] Verpoorte R, Choi YH, Kim HK. Metabolomics: will it stay? Phytochem Anal 2010; 21: 2–3

[23] Verpoorte R, Choi YH, Kim HK. Ethnopharmacology and systems biology: a perfect holistic match. J Ethnopharmacol 2005; 100: 53–56

[24] Li JJ. History of Drug Discovery. In: Li JJ, Corey EJ, eds. Drug Discovery: Practices, Processes, and Perspectives. Hoboken, NJ: John Wiley & Sons; 2013: 1–42

[25] Ullrich SF, Hagels H, Kayser O. Scopolamine: a journey from the field to clinics. Phytochem Rev 2017; 16: 333–353. doi:10.1007/s11101-016-9477-x

[26] Kreis W, Baron D, Stoll G. Biotechnologie der Arzneistoffe. Stuttgart, Germany: Deutscher Apotheker Verlag; 2001

[27] Wink M, Alfermann AW, Franke R, Wetterauer B, Distl M, Windhövel J, Krohn O, Fuss E, Garden H, Mohagheghzadeh A, Wildi E, Ripplinger P. Sustainable bioproduction of phytochemicals by plant *in vitro* cultures: anticancer agents. Plant Genet Resour Charact Util 2005; 3: 90–100

[28] Tabata M, Fujita Y. Production of Shikonin in Plant Cell Cultures. In: Zaitlin M, ed. Biotechnology in Plant Science. Cambridge, MA: Academic Press; 1985: 207–218

[29] Facchini PJ, Bohlmann J, Covello PS, De Luca V, Mahadevan R, Page JE, Ro DK, Sensen CW, Storms R, Martin VJJ. Synthetic biosystems for the production of high-value plant metabolites. Trends Biotechnol 2012; 30: 127–131

[30] Howat S, Park B, Oh IS, Jin YW, Lee EK, Loake GJ. Paclitaxel: biosynthesis, production and future prospects. N Biotechnol 2014; 31: 242–245

[31] Renneberg R. Biotech history: Yew trees, paclitaxel synthesis and fungi. Biotechnol J 2007; 2: 1207–1209

[32] Cusido RM, Onrubia M, Sabater-Jara AB, Moyano E, Bonfill M, Goossens A, Angeles Pedreño M, Palazon J. A rational approach to improving the biotechnological production of taxanes in plant cell cultures of *Taxus* spp. Biotechnol Adv 2014; 32: 1157–1167

[33] Muñoz-Torrero D, Cortés A, Mariño EL, Cusidó RM, Vidal H, Gallego A, Abdoli M, Palazón J. 6. Biotechnological production of taxanes: a molecular approach. Transw Res Netw 2013; 37661: 91–107

[34] Onrubia M, Cusidó RM, Ramirez K, Hernández-Vázquez L, Moyano E, Bonfill M, Palazon J. Bioprocessing of plant *in vitro* systems for the mass production of pharmaceutically important metabolites: paclitaxel and its derivatives. Curr Med Chem 2013; 20: 880–891

[35] Khosroushahi AY, Valizadeh M, Ghasempour A, Khosrowshahli M, Naghdibadi H, Dadpour MR, Omidi Y. Improved taxol production by combination of inducing factors in suspension cell culture of *Taxus baccata*. Cell Biol Int 2006; 30: 262–269

[36] Asghari G, Mostajeran A, Sadeghi H, Nakhai A. Effect of salicylic acid and silver nitrate on taxol production in *Taxus baccata*. J Med Plants 2012; 11: 74–82

[37] Weathers PJ, Towler MJ, Xu J. Bench to batch: advances in plant cell culture for producing useful products. Appl Microbiol Biotechnol 2010; 85: 1339–1351

[38] Malik S, Cusidó RM, Mirjalili MH, Moyano E, Palazón J, Bonfill M. Production of the anticancer drug taxol in *Taxus baccata* suspension cultures: a review. Process Biochem 2011; 46: 23–34

[39] Expósito O, Bonfill M, Onrubia M, Jané A, Moyano E, Cusidó RM, Palazón J, Piñol MT. Effect of taxol feeding on taxol and related taxane production in *Taxus baccata* suspension cultures. N Biotechnol 2009; 25: 252–259

[40] Qinghaosu Antimalaria Coordinating Research Group. Antimalaria studies on Qinghaosu. Chin Med J (Engl) 1979; 92: 811–816

[41] Ge H. Zhou Hou Bei Ji Fang. Tianjin, China: Tianjin Science and Technology Press; 2000

[42] Covello PS. Making artemisinin. Phytochemistry 2008; 69: 2881–2885

[43] Westfall PJ, Pitera DJ, Lenihan JR, Eng D, Woolard FX, Regentin R, Horning T, Tsuruta H, Melis DJ, Owens A, Fickes S, Diola D, Benjamin KR, Keasling JD, Leavell MD, McPhee DJ, Renninger NS, Newman JD, Paddon CJ. Production of amorphadiene in yeast, and its conversion to dihydroartemisinic acid, precursor to the antimalarial agent artemisinin. Proc Natl Acad Sci U S A 2012; 109: E111–E118

[44] Martin VJJ, Pitera DJ, Withers ST, Newman JD, Keasling JD. Engineering a mevalonate pathway in *Escherichia coli* for production of terpenoids. Nat Biotechnol 2003; 21: 796–802

[45] Turconi J, Griolet F, Guevel R, Oddon G, Villa R, Geatti A, Hvala M, Rossen K, Göller R, Burgard A. Semisynthetic artemisinin, the chemical path to industrial production. Org Process Res Dev 2014; 18: 417–422

[46] PATH. Sanofi and PATH announce the launch of large-scale production of semisynthetic artemisinin against malaria; 2013. Available at http://www.path.org/news/press-room/422/. Accessed May 17, 2018

[47] Bailey JE. Toward a science of metabolic engineering. Science 1991; 252: 1668–1675

[48] Staniek A, Bouwmeester H, Fraser PD, Kayser O, Martens S, Tissier A, van der Krol S, Wessjohann L, Warzecha H. Natural products – modifying metabolite pathways in plants. Biotechnol J 2013; 8: 1159–1171

[49] O'Connor SE. Engineering of secondary metabolism. Annu Rev Genet 2015; 49: 71–94

[50] Funahashi A, Matsuoka Y, Jouraku A, Morohashi M, Kikuchi N, Kitano H. CellDesigner 3.5: a versatile modeling tool for biochemical networks. Proc IEEE 2008; 96: 1254–1265

[51] Dasika MS, Maranas CD. OptCircuit: an optimization based method for computational design of genetic circuits. BMC Syst Biol 2008; 2: 24

[52] Dai Z, Liu Y, Zhang X, Shi M, Wang B, Wang D, Huang L, Zhang X. Metabolic engineering of *Saccharomyces cerevisiae* for production of ginsenosides. Metab Eng 2013; 20: 146–156

[53] Liu W, Stewart CN. Plant synthetic biology. Trends Plant Sci 2015; 20: 309–317

[54] Toya Y, Shimizu H. Flux analysis and metabolomics for systematic metabolic engineering of microorganisms. Biotechnol Adv 2013; 31: 818–826

[55] Dromms R, Styczynski M. Systematic applications of metabolomics in metabolic engineering. Metabolites 2012; 2: 1090–1122

[56] de la Parra J, Quave CL. Ethnophytotechnology: harnessing the power of ethnobotany with biotechnology. Trends Biotechnol 2017; 35: 802–806

[57] Pharmacompass. Natural product selling and pricing list. Available at https://www.pharmacompass.com. Accessed May 17, 2018

[58] Lim CG, Fowler ZL, Hueller T, Schaffer S, Koffas MAG. High-yield resveratrol production in engineered *Escherichia coli*. Appl Environ Microbiol 2011; 77: 3451–3460

[59] Fulzele DP, Heble MR. Large-scale cultivation of *Catharanthus roseus* cells: production of ajamalicine in a 20-l airlift bioreactor. J Biotechnol 1994; 35: 1–7

[60] Ramachandra Rao S, Ravishankar GA. Plant cell cultures: chemical factories of secondary metabolites. Biotechnol Adv 2002; 20: 101–153

[61] Ravishankar GA, Ramachandra Rao S. Biotechnological production of phyto-pharmaceuticals. J Biochem Mol Biol Biophys 2000; 4: 73–102

[62] Brochado AR, Matos C, Møller BL, Hansen J, Mortensen UH, Patil KR. Improved vanillin production in baker's yeast through *in silico* design. Microb Cell Fact 2010; 9: 84

[63] Koopman F, Beekwilder J, Crimi B, van Houwelingen A, Hall RD, Bosch D, van Maris AJA, Pronk JT, Daran JM. *De novo* production of the flavonoid naringenin in engineered *Saccharomyces cerevisiae*. Microb Cell Fact 2012; 11: 155

[64] Katz M, Durhuus T, Smits HP, Förster J. Production of metabolites. Patent WO2011147818; 2011

[65] Paddon CJ, Westfall PJ, Pitera DJ, Benjamin K, Fisher K, McPhee D, Leavell MD, Tai A, Main A, Eng D, Polichuk DR, Teoh KH, Reed DW, Treynor T, Lenihan J, Jiang H, Fleck M, Bajad S, Dang G, Dengrove D, Diola D, Dorin G, Ellens KW, Fickes S, Galazzo J, Gaucher SP, Geistlinger T, Henry R, Hepp M, Horning T, Iqbal T, Kizer L, Lieu B, Melis D, Moss N, Regentin R, Secrest S, Tsuruta H, Vazquez R, Westblade LF, Xu L, Yu M, Zhang Y, Zhao L, Lievense J, Covello PS, Keasling JD, Reiling KK, Renninger NS, Newman JD. High-level semi-synthetic production of the potent antimalarial artemisinin. Nature 2013; 496: 528–532

[66] Thodey K, Galanie S, Smolke CD. A microbial biomanufacturing platform for natural and semisynthetic opioids. Nat Chem Biol 2014; 10: 837–844