Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Plant Biology

# Heterologous production of plant-derived isoprenoid products in microbes and the application of metabolic engineering and synthetic biology

Yi Li and Blaine A Pfeifer

The value associated with plant-derived products has spurred efforts to engineer new production routes. One such option is heterologous biosynthesis which requires reconstitution of a biosynthetic pathway in a host that provides both innate and developed cellular advantages relative to the native producer. This review will summarize success to date in heterologously producing plant-derived isoprenoid products when using hosts such as *E. coli* and yeast. The article will also address the significant challenges that face such efforts, the approaches that have been used to overcome obstacles, and the tools of metabolic engineering and synthetic biology being applied both in the course of establishing heterologous biosynthesis and optimizing final production metrics.

**Addresses**
Department of Chemical and Biological Engineering, The State University of New York at Buffalo, Buffalo, NY 14260, United States

Corresponding author: Pfeifer, Blaine A (blainepf@buffalo.edu)

**Current Opinion in Plant Biology** 2014, **19**:8–13

This review comes from a themed issue on **Physiology and metabolism**

Edited by **Sarah E O'Connor** and **Thomas P Brutnell**

Available online XXX

1369-5266/$ – see front matter, © 2014 Elsevier Ltd. All rights reserved.

http://dx.doi.org/10.1016/j.pbi.2014.02.005

## Introduction

Plant-derived natural products have demonstrated notable value which has, in turn, driven recent bioengineering strategies to reconstitute biosynthesis through alternative production hosts [1••,2••]. Such strategies attempt to leverage several different forms of information and technology. For example, it is well-recognized that plant-derived compounds have shown promise as pharmaceuticals, agrochemicals, cosmetics, fine chemicals, and nutraceuticals [3,4]. However, the complexity of the compounds and their native production hosts has severely hampered efforts at rapid and large-scale production processes [5,6]. In response, scientific and engineering advances related to established fields such as metabolic engineering and emerging fields such as synthetic biology have offered new opportunities for improved access to plant-derived compounds and their strong potential [7,8].

Upon isolation of a compound from a plant host and confirmation of a desirable property, the process then begins towards dedicated production. The effort may take advantage of the native plant host [9]. Alternatively, upon elucidation of the compound's structure, a chemical synthesis strategy may be devised to produce the compound [10,11]. However, these approaches can be difficult to implement in an economic fashion due to issues such as the slow growth kinetics and low native titers from the plant source and the structural complexity associated with most plant-derived products.

As an alternative, one may turn to an experimentally convenient heterologous host that would offer the potential to reconstitute biosynthesis [12,13]. Success would address the challenges associated with the native plant and chemical synthetic production routes. However, with this approach comes a number of other significant challenges that must be addressed such as first, prerequisite genetic sequence information; second, proper design of the sequence information for active gene expression and biosynthesis; and third, improved production metrics that would allow for effective mass distribution. Though these hurdles are substantial, the heterologous biosynthetic approach is greatly aided by established and constantly evolving technology to address both reconstitution and scalable production. This concise review will provide an updated account of efforts to generate complex plant isoprenoid natural products using heterologous biosynthesis. A special emphasis will be placed on advances, including those associated with metabolic engineering and synthetic biology, that have allowed and improved success in such ventures.

## Heterologous biosynthesis

Table 1 summarizes heterologous production efforts for the isoprenoid plant-derived products lycopene, artemisinin, and paclitaxel. Key trends include first, each series of studies began with *E. coli* as a heterologous host, most-likely representing the technical advantages and ease of working with this host; second, a transition (particularly with artemisinin) to other heterologous hosts, most-likely representing some metabolic or biosynthetic advantage the new host provides; third, the progression from heterologous reconstitution to maximizing production, aided

**Table 1**

**Summary of efforts to produce plant-derived isoprenoid compounds (featuring lycopene, artemisinin, and paclitaxel) using heterologous biosynthetic strategies**

| Compound | Native plant | Heterologous host | Approach | Year | Reference |
|---|---|---|---|---|---|
| Lycopene | Tomato | *E. coli* | Plasmid-based expression of heterologous carotenoid biosynthesis genes from a photosynthetic bacterium | 1990 | [14] |
| | | *S. cerevisiae* | Plasmid-based expression of heterologous carotenoid genes under the control of native yeast promoters | 1994 | [15] |
| | | *C. utilis* | Plasmid-based expression of heterologous carotenoid genes combined with deletion of one ERG9 allele in a diploid strain together with over-expression of a truncated 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGR) gene | 1998 | [16] |
| | | *E. coli* | Overexpression of heterologous genes encoding 1-deoxy-D-xylose-5-phosphate synthase (DXS) | 1999 | [17] |
| | | *E. coli* | Directed evolution: mutant geranylgeranyl diphosphate synthase (GGPPS) gene combined with over-expression of the genes for isopentenyl diphosphate isomerase (IDI) and DXS | 2000 | [18] |
| | | *E. coli* | Balanced supply of biosynthetic precursors and altered flux distribution between glyceraldehyde 3-phosphate and pyruvate | 2001 | [19] |
| | | *E. coli* | Genome-wide stoichiometric flux balance analysis used in conjunction with combinatorial gene knockouts and global transposon library | 2005 | [20*] |
| | | *E. coli* | Combination of over-expression targets identified by screening of genomic libraries and knockout targets predicted by stoichiometric modeling | 2007 | [21] |
| | | *E. coli* | Multiplex Automated Genome Engineering (MAGE) used to modify 24 genetic components at once to optimize the 2C-methyl-D-erythritol-4-phosphate (MEP) pathway | 2009 | [22*] |
| | | *E. coli* | Chemically Inducible Chromosomal Evolution (CIChE) used to establish plasmid-free, high-gene-copy chromosomal expression | 2009 | [23] |
| | | *E. coli* | Flux scanning based on enforced objective flux (FSEOF) to identify gene amplification targets | 2010 | [24] |
| | | *E. coli* | High genetic stability during fermentation achieved by triclosan-induced chromosomal evolution | 2013 | [25] |
| | | *E. coli* | Vital intermediate phosphoenolpyruvate in the non-mevalonate pathway enriched by deleting the genes encoding the carbohydrate phosphotransferase system (PTS) to maximize lycopene and amorphadiene production | 2013 | [26] |
| Artemisinin (amorpha-4, 11-diene) | *Artemisia annua* | *E. coli* | Plasmid-based expression of heterologous *S. cerevisiae* genes encoding the mevalonate pathway and a codon-optimized amorpha-4,11-synthase (ADS) | 2003 | [27] |
| | | *S. cerevisiae* | Plasmid- and chromosomal-based expression of ADS gene from *A. annua* | 2006 | [28] |
| | | *S. cerevisiae* | An engineered mevalonate pathway and novel cytochrome P450 monooxygenase (CYP71AV1) from *A. annua* that performs a three-step oxidation | 2006 | [29] |
| | | *E. coli* | Two-phase partitioned bioreactor optimization for enhanced production | 2006 | [30] |
| | | *E. coli* | Balancing enzyme activity in a heterologous mevalonate pathway | 2007 | [31] |
| | | *Streptomyces avermitilis* | Biosynthetic amorphadiene pathway with minimal genome and synthetic genes optimized for expression | 2010 | [32] |
| | | *S. cerevisiae* | Co-engineering mitochondrion-targeted heterologous farnesyl diphosphate synthase (FPPS) and ADS with a truncated and deregulated HMGR | 2011 | [33] |
| | | *S. cerevisiae* | Demonstration of full artemisinic acid biosynthetic pathway and a semi-synthetic route to artemisinin | 2013 | [2••] |
| | | *Bacillus subtilis* | A two-promoter system coupled with protein translation engineering and systematic media optimization | 2013 | [34] |
| Paclitaxel (Taxadiene) | *Taxus brevifolia* | *E. coli* | Plasmid-based expression of genes encoding IDI, DXS, GGPPS, and taxadiene synthase (TXS) | 2001 | [35] |
| | | *S. cerevisiae* | Expression of the first portion of the paclitaxel biosynthetic pathway | 2006 | [36] |
| | | *S. cerevisiae* | Introduction of an alternate GGPPS from *Sulfolobus acidocaldarius* and a codon-optimized TXS from *Taxus chinensis* | 2008 | [37*] |
| | | *E. coli* | Modular-multivariate engineering of substrate and biosynthetic pathways | 2010 | [1••] |

significantly by emerging technology; fourth, improved production strategies featuring both direct (specific pathway alteration) and indirect (mutagenesis-based) methodologies. Directed strategies of improving cellular productivity consistently feature metabolic engineering and synthetic biology.

## The application of metabolic engineering and the introduction of synthetic biology

The simplest application of heterologous biosynthesis involves the direct transplantation of genetic components or pathways to the foreign host. However, this process will be complicated by differences in cellular backgrounds between the native and new hosts. Addressing such concerns saw the application of two fields particularly applicable to heterologous biosynthesis.

Metabolic engineering and synthetic biology have seen ready application to the heterologous production of plant-derived (and other) natural products from microbial systems. Aiding this development has been impressive technical breakthroughs in genome sequencing and annotation, DNA synthesis, chromosomal engineering, and analytical capabilities (including omics characterization methods) [38–42]. Returning to the context of reconstituted biosynthesis, oftentimes expression parameters must be adjusted for the new host. Promoters, regulation elements, chaperonins, and process conditions have all been used to help ensure successful prerequisite protein activity needed for biosynthesis [13,43]. As tools and technology began to mature and synthetic biology became more defined in the context of such efforts, approaches featured a range of promoters, operators, inducers, and gene designs (including the dedicated synthesis of genes for microbial codon usage) for the purpose of not only allowing for production but to begin the process of optimization [44]. Metabolic engineering saw early applications to heterologous biosynthesis in the designed provision of cellular substrates needed to ensure enzymatic activity [35,45]. Together, both fields found ready application to the heterologous reconstitution of foreign compounds through genetically-tractable microbial hosts (Table 1).

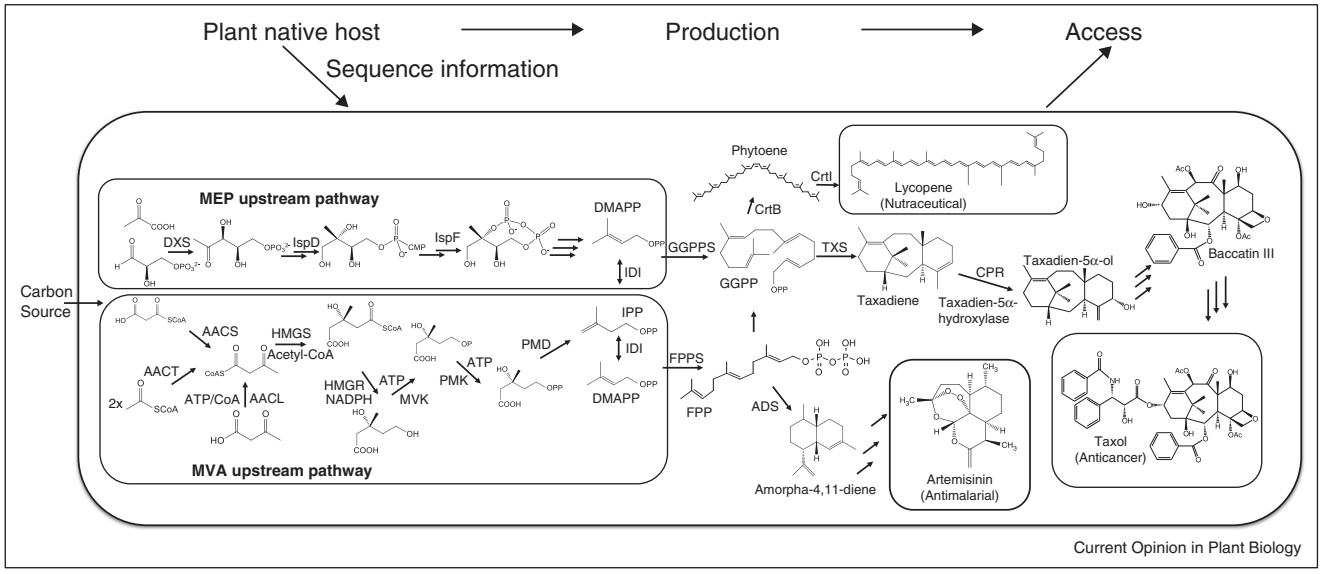
Once biosynthesis of a desired product has been accomplished, the same metabolic engineering and synthetic biology fields used to establish product formation can be dedicated to overproduction. This goal is always desirable to drive down eventual costs of the final compound. However, overproduction becomes critical for those compounds that support applications tethered to large populations (i.e. commodity products or therapies for developing nations). Metabolic engineering strategies often take a holistic view to the cellular system (aided by information provided by omics characterization) with the goal of maximizing carbon, reducing units, and energy for product formation [46]. As introduced above, synthetic biology tools can be applied to the individual components of a biosynthetic system, particularly when they have been identified as rate-limiting through metabolic engineering characterization.

With reference to Table 1, we will now highlight specific applications of metabolic engineering and synthetic biology in the context of plant-derived products from microbes. The three examples profiled share a common origin in the isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) precursors that must be provided in non-limiting amounts for maximal productivity. Metabolic engineering was applied to the two possible options natively capable of precursor supply: the mevalonate (MVA) and non-mevalonate (MEP) pathways (Figure 1). Interestingly, these efforts saw the successful transplantation of the MVA pathway, a staple of eukaryotic metabolism, to the *E. coli* cellular background natively harboring non-mevalonate metabolism. Precursor supply then prefaced downstream poly-isoprene biosynthesis. Additional metabolic engineering approaches (that spanned both experimental and computational themes) saw initial and very successful application to lycopene biosynthesis. In the cases of artemisinin and paclitaxel, however, a dedicated terpene synthase was required to begin the biosynthetic steps to final products. Initiating production was challenging because of the foreign nature of the transplanted genes, made more difficult because of their plant origins. Codon optimization, using the rapidly developing gene synthesis protocols fundamental to synthetic biology, was thus employed in providing successful gene expression. Complicating the process further were the next series of reactions crucial to artemisinin and paclitaxel formation which required plant P450 enzymes. These genes contained cellular localization sequences that were particularly irrelevant in bacterial cellular backgrounds. As such, additional gene synthesis and expression tools played important roles in providing bacterial expression and activity. Alternatively, yeast systems were utilized for P450 expression to leverage cellular features that more closely resembled plant cells. Success in both bacterial and yeast host systems has set the stage for additional efforts in reconstitution and has also led to the g/L production of paclitaxel and artemisinin intermediates. Of note, the semi-synthetic heterologous production-based process for artemisinin has reached commercial application.

## Future directions

The ultimate objective in heterologous production of plant-derived products is the rapid and robust access to the desired compound. Initiating the process requires a genetic pathway to the compound of interest. This pathway may already exist or there may be the option of rationally building the pathway from genes/enzymes with known functionality [47,48]. Genomic and metabolic

**Figure 1**




Plant-derived isoprenoid heterologous biosynthetic pathway elucidation and reconstruction process featuring lycopene, artemisinin, and paclitaxel. IspD: 4-diphosphocytidyl-2-*C*-methyl-D-erythritol synthase; IspF: 2-*C*-methyl-D-erythritol 2,4-cyclodiphosphate synthase; CrtB: phytoene synthase; CrtI: phytoene desaturase; CPR: taxadien-5α hydroxylase redox partner, *Taxus* cytochrome P450 reductase; AACS: acetoacetyl-CoA synthase; AACT: acetoacetyl-CoA thiolase; AACL: acetoacetyl-CoA ligase; HMGS: hydroxymethylglutaryl-CoA synthase; MVK: mevalonate kinase; PMK: phosphomevalonate kinase; PMD: mevalonate diphosphate decarboxylase.

knowledge will allow pathway identification, greatly aided by the ever-increasing improvements in sequencing technology and metabolic deduction of the desired pathways/compounds.

Once identified, the pathways must be constructed for expression. In this capacity, synthetic biology will play a crucial role. Design rules are emerging for the genetic parameters accompanying the expression of entire pathways. These will continue to develop, aided by improvements in gene synthesis towards the streamlined construction of pathways capable of heterologous biosynthesis.

The foreign host choice is also an evolving component of the heterologous biosynthetic scheme and is a function of experience, technical advantages, and appropriate utility. As one example, *E. coli*, perhaps the most technically-advanced of heterologous host choices, has been used extensively for plant-derived heterologous production efforts. However, efforts that require P450 gene expression and protein activity often rely on yeast hosts [49]. The 'correct' choice is a matter of balancing advantages and challenges associated with these and other host options. Alternatively, there are options that may include step-wise heterologous transformations that utilize both hosts (*E. coli* in one transformation followed or preceded by yeast in other transformations). More recently, the concept of host generation has been imagined, in which a new host is built from the bottom up, dictated by a synthetic genome that is designed for both cellular function and heterologous production [50•].

Establishment of heterologous biosynthesis, depending on the initial production levels, is often insufficient. Instead, improvements to production metrics must be made. In this capacity, metabolic engineering tools that include both computational and experimental means of quantifying and redirecting cellular fluxes to the desired compound are utilized to improve specific productivity [1••]. Process engineering can then be used to improve volumetric productivities through approaches that include vessel scaling (and the use of sophisticated bioreactor designs and processes) and medium optimization (including statistical design of experiment approaches). The end goal of each level of engineering is efficient and maximized production of the new product, with an overall objective to seamlessly access and translate the value of plant-derived products to application and widespread utilization.

## Acknowledgements

Work related to isoprenoid and general themes in heterologous biosynthesis has been graciously supported by funding from the NIH, NSF, Milheim Foundation, and DoE.

## References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. •• Ajikumar PK, Xiao WH, Tyo KEJ, Wang Y, Simeon F, Leonard E, Mucha O, Phon TH, Pfeifer B, Stephanopoulos G: **Isoprenoid pathway optimization for taxol precursor overproduction in *Escherichia coli***. *Science* 2010, **330**:70-74.

This article presents a comprehensive metabolic engineering effort to balance upstream (in this case, the non-mevalonate pathway) and downstream modules of early Taxol intermediate production. When coupled to process engineering strategies (namely, controlled bioreactor operations), titers reached undrecedented levels for the early intermediates produced.

2. •• Paddon CJ, Westfall PJ, Pitera DJ, Benjamin K, Fisher K, McPhee D, Leavell MD, Tai A, Main A, Eng D *et al.*: **High-level semi-synthetic production of the potent antimalarial artemisinin**. *Nature* 2013, **496**:528-532.

This article presents a commercial semi-synthetic route to artemisinin with the artemisinic acid precursor produced at 25 g/L through a combination of synthetic biology and metabolic engineering. The work presents an impressive example of the successful application of heterologous biosynthetic themes.

3. Raskin I, Ribnicky DM, Komarnytsky S, Ilic N, Poulev A, Borisjuk N, Brinker A, Moreno DA, Ripoll C, Yakoby N *et al.*: **Plants and human health in the twenty-first century**. *Trends Biotechnol* 2002, **20**:522-531.

4. Itokawa H, Morris-Natschke SL, Akiyama T, Lee KH: **Plant-derived natural product research aimed at new drug discovery**. *J Nat Med* 2008, **62**:263-280.

5. Patil RA, Kolewe ME, Roberts SC: **Cellular aggregation is a key parameter associated with long term variability in paclitaxel accumulation in *Taxus* suspension cultures**. *Plant Cell Tissue Organ Cult* 2013, **112**:303-310.

6. Smetanska I: **Production of secondary metabolites using plant cell cultures**. *Adv Biochem Eng Biotechnol* 2008, **111**:187-228.

7. Mitchell W: **Natural products from synthetic biology**. *Curr Opin Chem Biol* 2011, **15**:505-515.

8. Keasling JD: **Synthetic biology and the development of tools for metabolic engineering**. *Metab Eng* 2012, **14**:189-195.

9. Wilson SA, Roberts SC: **Recent advances towards development and commercialization of plant cell culture processes for the synthesis of biomolecules**. *Plant Biotechnol J* 2012, **10**:249-268.

10. Danishefsky SJ, Masters JJ, Young WB, Link JT, Snyder LB, Magee TV, Jung DK, Isaacs RCA, Bornmann WG, Alaimo CA *et al.*: **Total synthesis of baccatin III and taxol**. *J Am Chem Soc* 1996, **118**:2843-2859.

11. Baloglu E, Kingston DG: **A new semisynthesis of paclitaxel from baccatin III**. *J Nat Prod* 1999, **62**:1068-1071.

12. Ongley SE, Bian X, Neilan BA, Muller R: **Recent advances in the heterologous expression of microbial natural product biosynthetic pathways**. *Nat Prod Rep* 2013, **30**:1121-1138.

13. Zhang H, Boghigian BA, Armando J, Pfeifer BA: **Methods and options for the heterologous production of complex natural products**. *Nat Prod Rep* 2011, **28**:125-151.

14. Misawa N, Nakagawa M, Kobayashi K, Yamano S, Izawa Y, Nakamura K, Harashima K: **Elucidation of the *Erwinia uredovora* carotenoid biosynthetic pathway by functional analysis of gene products expressed in *Escherichia coli***. *J Bacteriol* 1990, **172**:6704-6712.

15. Yamano S, Ishii T, Nakagawa M, Ikenaga H, Misawa N: **Metabolic engineering for production of beta-carotene and lycopene in *Saccharomyces cerevisiae***. *Biosci Biotechnol Biochem* 1994, **58**:1112-1114.

16. Shimada H, Kondo K, Fraser PD, Miura Y, Saito T, Misawa N: **Increased carotenoid production by the food yeast *Candida utilis* through metabolic engineering of the isoprenoid pathway**. *Appl Environ Microbiol* 1998, **64**:2676-2680.

17. Harker M, Bramley PM: **Expression of prokaryotic 1-deoxy-D-xylulose-5-phosphatases in *Escherichia coli* increases carotenoid and ubiquinone biosynthesis**. *FEBS Lett* 1999, **448**:115-119.

18. Wang C, Oh MK, Liao JC: **Directed evolution of metabolically engineered *Escherichia coli* for carotenoid production**. *Biotechnol Prog* 2000, **16**:922-926.

19. Farmer WR, Liao JC: **Precursor balancing for metabolic engineering of lycopene production in *Escherichia coli***. *Biotechnol Prog* 2001, **17**:57-61.

20. • Alper H, Miyaoku K, Stephanopoulos G: **Construction of lycopene-overproducing *E. coli* strains by combining systematic and combinatorial gene knockout targets**. *Nat Biotech* 2005, **23**:612-616.

In this work, both directed and non-directed metabolic engineering methods were applied to the improvement of lycopene formation. The results emphasize that both routes stand as complementary ways of improving heterologous production metrics.

21. Jin YS, Stephanopoulos G: **Multi-dimensional gene target search for improving lycopene biosynthesis in *Escherichia coli***. *Metab Eng* 2007, **9**:337-347.

22. • Wang HH, Isaacs FJ, Carr PA, Sun ZZ, Xu G, Forest CR, Church GM: **Programming cells by multiplex genome engineering and accelerated evolution**. *Nature* 2009, **460**:894-898.

The demonstration of MAGE technology to simultaneously engineering gene expression across the bacterial chromosome. This and similar technology are increasing the options and throughput available for engineering heterologous biosynthesis reconstitution and optimization.

23. Tyo KE, Ajikumar PK, Stephanopoulos G: **Stabilized gene duplication enables long-term selection-free heterologous pathway expression**. *Nat Biotechnol* 2009, **27**:760-765.

24. Choi HS, Lee SY, Kim TY, Woo HM: **In silico identification of gene amplification targets for improvement of lycopene production**. *Appl Environ Microbiol* 2010, **76**:3097-3105.

25. Chen YY, Shen HJ, Cui YY, Chen SG, Weng ZM, Zhao M, Liu JZ: **Chromosomal evolution of *Escherichia coli* for the efficient production of lycopene**. *BMC Biotechnol* 2013, **13**:1472-6750.

26. Zhang C, Chen X, Zou R, Zhou K, Stephanopoulos G, Too HP: **Combining genotype improvement and statistical media optimization for isoprenoid production in *E. coli***. *PLoS ONE* 2013, **8**:0075164.

27. Martin VJJ, Pitera DJ, Withers ST, Newman JD, Keasling JD: **Engineering a mevalonate pathway in *Escherichia coli* for production of terpenoids**. *Nat Biotech* 2003, **21**:796-802.

28. Lindahl A-L, Olsson M, Mercke P, Tollbom Ö, Schelin J, Brodelius M, Brodelius P: **Production of the artemisinin precursor amorpha-4,11-diene by engineered *Saccharomyces cerevisiae***. *Biotechnol Lett* 2006, **28**:571-580.

29. Ro DK, Paradise EM, Ouellet M, Fisher KJ, Newman KL, Ndungu JM, Ho KA, Eachus RA, Ham TS, Kirby J *et al.*: **Production of the antimalarial drug precursor artemisinic acid in engineered yeast**. *Nature* 2006, **440**:940-943.

30. Newman JD, Marshall J, Chang M, Nowroozi F, Paradise E, Pitera D, Newman KL, Keasling JD: **High-level production of amorpha-4,11-diene in a two-phase partitioning bioreactor of metabolically engineered *Escherichia coli***. *Biotechnol Bioeng* 2006, **95**:684-691.

31. Pitera DJ, Paddon CJ, Newman JD, Keasling JD: **Balancing a heterologous mevalonate pathway for improved isoprenoid production in *Escherichia coli***. *Metab Eng* 2007, **9**:193-207.

32. Komatsu M, Uchiyama T, Omura S, Cane DE, Ikeda H: **Genome-minimized *Streptomyces* host for the heterologous expression of secondary metabolism**. *Proc Natl Acad Sci U S A* 2010, **107**:2646-2651.

33. Farhi M, Marhevka E, Masci T, Marcos E, Eyal Y, Ovadis M, Abeliovich H, Vainstein A: **Harnessing yeast subcellular compartments for the production of plant terpenoids**. *Metab Eng* 2011, **13**:474-481.

34. Zhou K, Zou R, Zhang C, Stephanopoulos G, Too H-P: **Optimization of amorphadiene synthesis in *Bacillus subtilis* via transcriptional, translational, and media modulation**. *Biotechnol Bioeng* 2013, **110**:2556-2561.

35. Huang Q, Roessner CA, Croteau R, Scott AI: **Engineering *Escherichia coli* for the synthesis of taxadiene, a key intermediate in the biosynthesis of taxol**. *Bioorg Med Chem* 2001, **9**:2237-2242.

36. DeJong JM, Liu YL, Bollon AP, Long RM, Jennewein S, Williams D, Croteau RB: **Genetic engineering of Taxol biosynthetic genes in *Saccharomyces cerevisiae***. *Biotechnol Bioeng* 2006, **93**: 212-224.

37. • Engels B, Dahm P, Jennewein S: **Metabolic engineering of taxadiene biosynthesis in yeast as a first step towards Taxol (Paclitaxel) production**. *Metab Eng* 2008, **10**:201-206.
This study describes a metabolic engineering strategy within yeast for the productoin of taxadiene. Given the complexities of the remaining Taxol pathway, future utilization of yeast is a likely possibilty for next-step intermediate heterologous production.

38. Carr PA, Church GM: **Genome engineering**. *Nat Biotechnol* 2009, **27**:1151-1162.

39. Fuller CW, Middendorf LR, Benner SA, Church GM, Harris T, Huang X, Jovanovich SB, Nelson JR, Schloss JA, Schwartz DC *et al.*: **The challenges of sequencing by synthesis**. *Nat Biotechnol* 2009, **27**:1013-1023.

40. Patti GJ, Yanes O, Siuzdak G: **Innovation: metabolomics: the apogee of the omics trilogy**. *Nat Rev Mol Cell Biol* 2012, **13**:263-269.

41. Kaltashov IA, Bobst CE, Abzalimov RR, Wang G, Baykal B, Wang S: **Advances and challenges in analytical characterization of biotechnology products: mass spectrometry-based approaches to study properties and behavior of protein therapeutics**. *Biotechnol Adv* 2012, **30**:210-222.

42. Blazeck J, Alper HS: **Promoter engineering: recent advances in controlling transcription at the most fundamental level**. *Biotechnol J* 2013, **8**:46-58.

43. Wang HH, Kim H, Cong L, Jeong J, Bang D, Church GM: **Genome-scale promoter engineering by coselection MAGE**. *Nat Methods* 2012, **9**:591-593.

44. Enyeart PJ, Ellington AD: **Synthetic biology: a yeast for all reasons**. *Nature* 2011, **477**:413-414.

45. Pfeifer BA, Admiraal SJ, Gramajo H, Cane DE, Khosla C: **Biosynthesis of complex polyketides in a metabolically engineered strain of *E. coli***. *Science* 2001, **291**:1790-1792.

46. Woolston BM, Edgar S, Stephanopoulos G: **Metabolic engineering: past and future**. *Annu Rev Chem Biomol Eng* 2013, **4**:259-288.

47. Prather KLJ, Martin CH: **De novo biosynthetic pathways: rational design of microbial chemical factories**. *Curr Opin Biotechnol* 2008, **19**:468-474.

48. Dietrich JA, Yoshikuni Y, Fisher KJ, Woolard FX, Ockey D, McPhee DJ, Renninger NS, Chang MC, Baker D, Keasling JD: **A novel semi-biosynthetic route for artemisinin production using engineered substrate-promiscuous P450(BM3)**. *ACS Chem Biol* 2009, **4**:261-267.

49. 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, **11**:146-156.

50. • Gibson DG, Glass JI, Lartigue C, Noskov VN, Chuang RY, Algire MA, Benders GA, Montague MG, Ma L, Moodie MM *et al.*: **Creation of a bacterial cell controlled by a chemically synthesized genome**. *Science* 2010, **329**:52-56.
This work indicates a provocative future direction for the heterologous biosynthetic field in which a cellular platform is designed and generated for the express purpose of producing a targeted compound.