Chemical Engineering Science ▮ (▮▮▮▮) ▮▮▮–▮▮▮

Contents lists available at SciVerse ScienceDirect

Chemical Engineering Science

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

Genome-scale *in silico* modeling and analysis for designing synthetic terpenoid-producing microbial cell factories

Bevan Kai-Sheng Chung [a,b,c], Meiyappan Lakshmanan [b], Maximilian Klement [b,c], Bijayalaxmi Mohanty [b], Dong-Yup Lee [a,b,c,*]

[a] NUS Graduate School for Integrative Sciences and Engineering, National University of Singapore, 28 Medical Drive, #05-01, Singapore 117456, Singapore
[b] Department of Chemical and Biomolecular Engineering, National University of Singapore, 4 Engineering Drive 4, Singapore 117576, Singapore
[c] Bioprocessing Technology Institute, Agency for Science, Technology and Research (A*STAR), 20 Biopolis Way, #06-01, Centros, Singapore 138668, Singapore

HIGHLIGHTS

- Developed in silico rational framework for synthetic terpenoid production.
- Updated metabolic model of A. thaliana to describe terpenoid biosynthesis.
- Analyzed heterologous expression of A. thaliana pathways in microbial systems.
- Proposed novel in silico method of cofactor modification analysis.
- Improve terpenoid yield by modifying enzyme cofactor specificity.

ARTICLE INFO

Article history:
Received 9 May 2012
Received in revised form
14 August 2012
Accepted 11 September 2012

Keywords:
Synthetic biology
Terpenoid
Genome-scale metabolic model
Arabidopsis
Microbial cell factories
Cofactor modification analysis

ABSTRACT

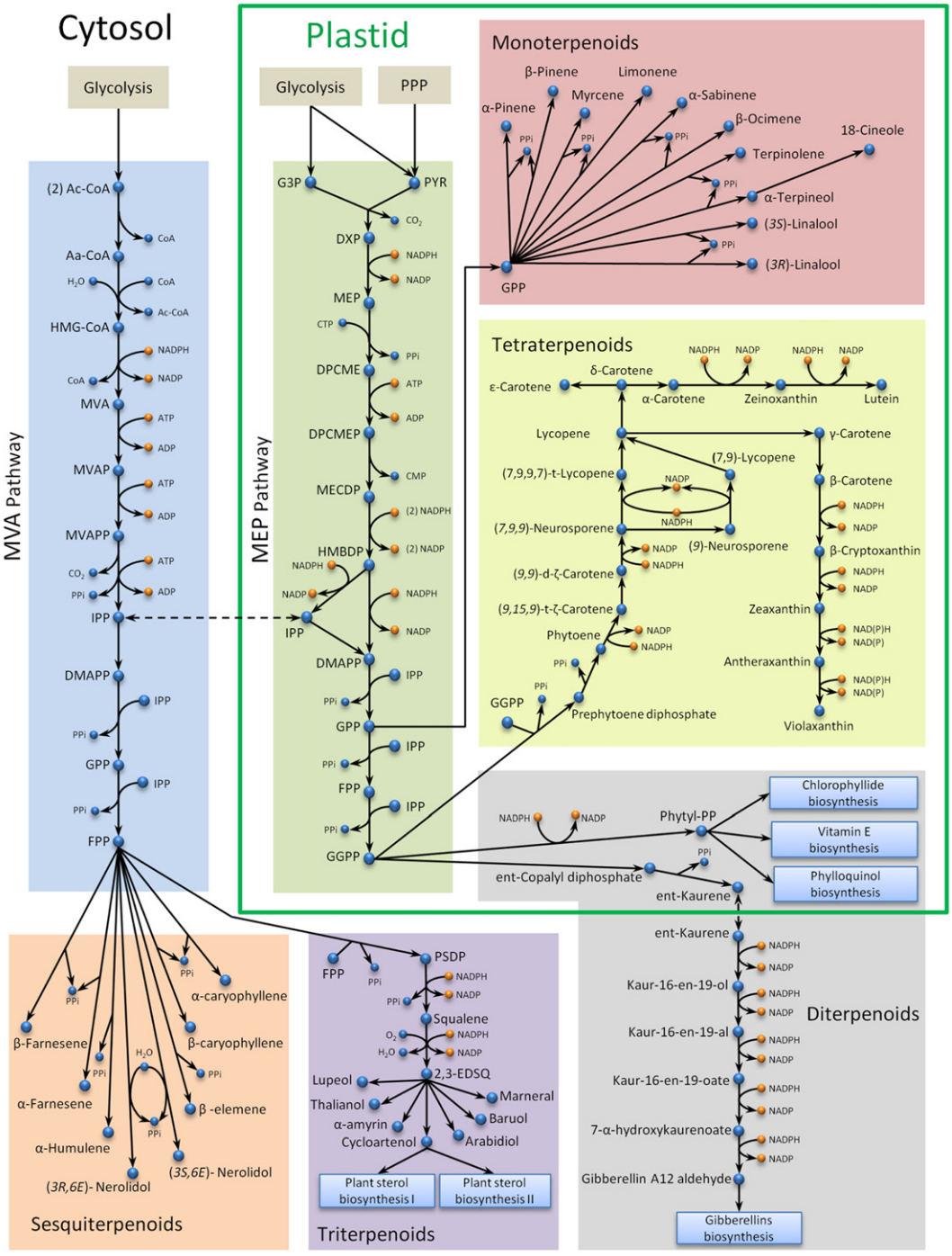
Terpenoids are a large and diverse group of plant secondary metabolites with important applications in the pharmaceutical, cosmetic and food industries. However, low yields obtained from natural plant sources necessitate the search for alternative ways to increase the throughput of terpenoid production. Thus, fast-growing microbial systems, such as *Escherichia coli* and *Saccharomyces cerevisiae*, can be genetically engineered to achieve high productivity by systematically designing terpenoid synthetic routes and improving its precursor, isopentenyl diphosphate (IPP) production. To this end, we develop an *in silico* model-based rational framework where the cellular metabolism in natural terpenoid producer is analyzed to provide a basis for designing its synthetic pathways in microbial hosts. At the outset, we updated the genome-scale *Arabidopsis thaliana* metabolic model to characterize optimal metabolic utilization patterns that were subsequently incorporated into the *in silico* models of microbial hosts for improving terpenoid yield. We also developed a novel computational approach, cofactor modification analysis (CMA), to tackle potential limitations in terpenoid production caused by suboptimal balance of the different redox cofactors. The enzyme targets identified by CMA can potentially lead to better metabolic engineering strategies for enhancing terpenoid production in microbial systems.

1. Introduction

Plant secondary metabolites, such as alkaloids, terpenoids and phenols, play an essential role in the defense against damage by herbivores (Buchanan et al., 2000; Keeling and Bohlmann, 2006). Among these secondary metabolites, terpenoids constitute the largest class of natural organic compounds with about 25,000 chemical structures being reported (Gershenzon and Dudareva, 2007). Such diverse terpenoids can be synthesized from five-carbon isoprene precursors, isopentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP) through a combination of biosynthetic pathways, thereby forming many multicyclic structures, comprising different carbon skeletons attached with various functional groups (Carretero-Paulet et al., 2002; Rodriguez-Concepcion and Boronat, 2002; Withers and Keasling, 2007). The terpenoids are classified according to the number of isoprene units (Fig. 1). The wide range of biological properties unique to each terpenoid species have been largely exploited for perfume manufacture and therapeutic applications to formulate

* Corresponding author at: Department of Chemical and Biomolecular Engineering, National University of Singapore, 4 Engineering Drive 4, Singapore 117576, Singapore. Tel.: +65 6516 6907; fax: +65 6779 1936.
E-mail address: cheld@nus.edu.sg (D.-Y. Lee).

0009-2509/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.ces.2012.09.006



Class of terpenoid	No. of IPP units	No. of C atoms	General structure
Monoterpenoids	2	10	
Sesquiterpenoids	3	15	
Diterpenoids	4	20	
Triterpenoids	6	30	
Tetraterpenoids	8	40	

Fig. 1. Terpenoid biosynthesis pathways. The biosynthesis of terpenoids occurs via linear pathways which branch out from the precursors formed by the MEP and/or MVA pathways. The pathway information is obtained from online database as described in Section 3.1. Metabolite abbreviations: Aa-CoA, acetoacetyl-CoA; Ac-CoA, acetyl-CoA; DMAPP, dimethylallyl diphosphate; DPCME, 4-diphosphocytidyl-2-C-methylerythritol; DPCMEP, 4-diphosphocytidyl-2-C-methyl-D-erythritol 2-phosphate; DXP, 1-deoxy-D-xylulose 5-phosphate; FPP, farnesyl diphosphate; G3P, glyceraldehyde-3-phosphate; GGPP, geranylgeranyl diphospahate; GPP, geranyl diphosphate; HMBDP, (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate; HMG-CoA, hydroxymethylglutaryl-CoA; IPP, isopentenyl diphosphate; MECDP, 2-C-methyl-D-erythritol 2,4-cyclopyrophosphate; MEP, 2-C-methyl-D-erythritol 4-phosphate; MVA, mevalonic acid; MVAP, mevalonate-5-phosphate; MVAPP, mevalonate-5-diphosphate; and PYR, pyruvate.