**Microbial discovery and production of terpene synthases and isoprenoids**

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**Abstract**

Isoprenoids, or terpenoids, constitute possibly the largest group of natural products (>75,000). Structural diversity of terpenoids contributes to wide applications ranging from pharmaceuticals (*e.g.,* artemisinin), nutraceuticals (*e.g.,* astaxanthin), flavors and fragrances (*e.g.,* linalool), polymer molecules (*e.g.*, isoprene) and biofuels (*e.g.,* farnesene). Unlike plant terpenes that are well studied, fungal terpenes and their synthases remain largely untapped. In collaboration with a German group, we have developed an integrated platform for discovery of novel fungal terpene synthases (TPSs). Coupling bioinformatics (all-by-all blast and active site prediction) and experiments (SPME-GCMS), we have successfully predicted several unique clusters of putative isofunctional TPSs (e.g. protoilludene, viridiflorol) and validated by experiments. In addition to terpene synthase discovery, we also developed a multidimensional heuristic process (MHP) to efficiently synthesize these molecules in *Escherichia coli*. Built on statistical analysis and modular metabolic engineering methods, MHP is a focused and systematic approach to optimize the performance of biosynthetic pathways, especially effective on complex systems (>10 genes). MHP adopts 'modular design' that significantly reduces experimental workload yet without losing the flexibility by simultaneously control different functional dimensions (transcription, translation and enzymes). As proof-of-concept examples, we effectively enhanced the total biosynthesis of the 15-step heterologous biosynthetic route of two tetraterpenoid (C40), astaxanthin, at 320 mg/L and lycopene (1.5 g/L); a monoterpenoid (C10), linalool (0.5 g/L); and two sesquiterpenoids (C15), viridiflorol (~25.7 g/L) and amorphadiene (30 g/L). Evidently, MHP is generally applicable for different metabolic pathways and products.

**Speaker's biography**

Dr Congqiang ZHANG (Simon) received his undergraduate and master training in chemical engineering, Tianjin University, China. He then continued with his PhD training in a joint programme in Chemical and Pharmaceutical Engineering, Singapore-MIT Alliance (SMA) between National University of Singapore (NUS) and Massachusetts Institute of Technology (MIT). He studied metabolic engineering and was supervised under Prof Too Heng-Phon, NUS and Prof Gregory Stephanopoulos, MIT. After graduation in 2013, he worked as postdoc in NUS, leading an innovation grant of developing the industrial production of artemisinin and carotenoids, funded by Singapore-MIT Alliance for Research and Technology (SMART). In 2015, he is recruited as the second employees of Biotransformation Innovation Platform (BioTrans), Agency for science, technology and research (A*STAR), Singapore. As a pioneer and core member, he assisted the cultural, structural building and lab setup of BioTrans. He is now leading a team in BioTrans working on multiple academic and industrial projects and initiating international collaborations between Singapore and Chinese, German and French universities. His expertise is metabolic engineering, synthetic biology, enzyme discovery and engineering, especially industrial biotechnology of microorganisms. He has published in top journals of metabolic engineering and synthetic biology and filed a few patents on industrial production of terpenoids including carotenoids and apocarotenoids and discovery/engineering of novel terpene synthases. He serves as an executive member of BioEnergy Society of Singapore and an active reviewer of many prestigious journals in biotechnology, metabolic engineering and synthetic biology.

**Brief CV**

**Congqiang Zhang, Ph.D.**

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**Education:**

BS Chemical engineering, Tianjin University, China, 2007

MS Chemical engineering, Tianjin University, China, 2009

Ph.D. Chemical and pharmaceutical engineering, National University of Singapore (Singapore-MIT Alliance), Singapore, 2014,

**Professional Career:**

2014-2015: National University of Singapore, postdoc.

2015-2018: Agency for science, technology and research, research scientist

2018-date: Agency for science, technology and research, research scientist and team lead

**Research Interests:**

1. Metabolic engineering and synthetic biology
2. Enzyme discovery and engineering
3. Industrial biotechnology
4. Food science and technology

**Selected publications (* corresponding author)**

1. Shukal, S., Chen, X. & ZHANG, C*. Systematic engineering for high-yield production of viridiflorol and amorphadiene in auxotrophic Escherichia coli. **Metabolic Engineering** 55, 170–178 (2019).
2. Chen, X.*, Shukal, S. & ZHANG, C. Integrating Enzyme and Metabolic Engineering Tools for Enhanced α-Ionone Production. **J. Agric. Food Chem**. acs.jafc.9b00860 (2019).
3. ZHANG, C.* & Too, H.-P*. Revalorizing Lignocellulose for the Production of Natural Pharmaceuticals and Other High Value Bioproducts. **Curr. Med. Chem.** 26, 2475–2484 (2019).

4. ZHANG, C.*, Seow, V. Y., Chen, X. & Too, H.-P*. Multidimensional heuristic process for high-yield production of astaxanthin and fragrance molecules in Escherichia coli. **Nat Commun** 9, 1858 (2018).
5. ZHANG, C.*, Chen, X., Lindley, N. D. & Too, H.-P*. A 'plug-n-play' modular metabolic system for the production of apocarotenoids. **Biotechnol. Bioeng.** 115, 174–183 (2018).
6. Chen, X., ZHANG, C. & Too, H.-P*. Multienzyme Biosynthesis of Dihydroartemisinic Acid. **Molecules** 22, (2017).
7. Chen, X., ZHANG, C., Zou, R., Stephanopoulos, G. & Too, H.-P*. In vitro metabolic engineering of amorpha-4,11-diene biosynthesis at enhanced rate and specific yield of production. **ACS. Synth. Biol.** acssynbio.6b00377 (2017). doi:10.1021/acssynbio.6b00377
8. ZHANG, C., Chen, X., Stephanopoulos, G. & Too, H.-P*. Efflux transporter engineering markedly improves amorphadiene production in Escherichia coli. **Biotechnol. Bioeng.** 113, 1755–1763 (2016).
9. ZHANG, C., Zou, R., Chen, X., Stephanopoulos, G. & Too, H.-P*. Experimental design-aided systematic pathway optimization of glucose uptake and deoxyxylulose phosphate pathway for improved amorphadiene production. **Appl Microbiol Biotechnol** 99, 3825–3837 (2015).
10. 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.** 110, 2556–2561 (2013).
11. ZHANG, C. et al. Combining genotype improvement and statistical media optimization for isoprenoid production in E. coli. **PLoS ONE** 8, e75164 (2013).