

Sesterterpene biosynthesis: from marine fungus *Aspergillus* sp. to *E. coli*

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Abstract

Terpenoids are the most diverse family of natural products. The artemisinin and paclitaxel are well known antimalarial and anticancer terpenoids drugs, respectively. A mangrove Ascomycota fungus *Aspergillus ustus* 094102 produces a series of biological active terpenoids. Whole genome sequence of strain 094102 facilitates systematically unveiling the biosynthetic pathway of sesterterpene ophiobolins. Using *in vivo* genetic manipulation, we obtained 5 gene cluster knock out mutants, which the yield of ophiobolin were changed and one completely abolished. Involvement of 5 gene clusters in ophiobolin synthesis was confirmed by investigation on the 5 key terpene synthesis enzymes of each gene cluster, either by gene deletion and complementation or protein *in vitro* function verification. The results ascertained that ophiobolin skeleton biosynthesis was involved by 5 gene clusters, which related to C15, C20, C25, and C30 terpenoids biosynthesis. Using synthetic biology approach, we constructed the *E. coli* chassis to produce the ophiobolins. The above 4 terpene synthase genes related to ophiobolin production in strain 094102, were individually or combinatorially overexpressed with Au8003 to mimic the original fungal biosynthesis. The biosynthesis of ophiobolin scaffold was increased by short-chain terpene synthases (C15 and C20), among which the C15 synthase gene contributed the highest yield of 82.76 mg/L at 96 h; the multi-gene combinatorial results suggested that cyclization may be a rate-limiting step. Further protein engineering including fusion tags and phylogenetically-based mutations on the rate-limiting cyclization part of the bifunctional terpene synthase enabled a further yield improvement (>150 mg/L at 96 h) in shake flasks. These multiple approaches for sesterterpene skeleton production using engineered *E. coli* may be applicable for cost-effective, high-yield productions of ophiobolins and other compounds synthesized by bifunctional terpene synthases.

Speaker's biography

Dr Kui Hong is a Professor in School of Pharmaceutical Sciences, Wuhan University, Wuhan, China. She is the "New century excellent talents" in support of Ministry of education plan. She has studied microbiology at Wuhan University, South China College of Tropical Crops, Nanjing Agriculture University and Tsinghua University, where she obtained degrees of BS, MS and Ph D. Since 2001, her research is major on marine microbial drug discovery, study on collection and evaluation of marine microbial drug resources (culture, compounds and genes), especially from special marine environments of mangrove, deep sea and polar region; biosynthesis of the marine microbial bioactive secondary metabolites such as terpenoids to increase the yield for drug discovery. She has been in charge of or take part in the national projects of the NSFC, "863", "973" and the international research projects of the EU"FP7" and China- Thai collaboration. She is the committee member of Chinese Microbiology Society and Chinese Biochemistry Society, and belongs to the editor board of the international journal of "Marine Drugs". She possesses 19 national and 1 PCT patents. She published about 100 international publications. She is the chief scientist of the "National Key R&D Program of China" "High efficient discovery and modification of marine microbial drug candidate".



Brief CV

Kui Hong, Ph.D.

“New century excellent talents” in support of Ministry of education plan

Wuhan University School of Pharmaceutical Sciences

Education:

BS Microbiology, Wuhan University, China, 1988

MS Microbiology, South China College of Tropical Crops, China, 1991

Ph.D. Microbiology, Tsinghua University and Nanjing Agricultural University, China, 1998

Professional Career:

12/10-Present: Professor, Wuhan University School of Pharmaceutical Sciences_

07/13-09/13: Visiting Scientist, Aberdeen University, UK

06/09-06/09: Visiting Scientist, Plymouth Marine Lab, UK

06/00-12/10: Professor, Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Biosciences and Biotechnology

09/05-11/05: supported by Royal Society of UK, KC Wong fellowship, Visiting in Newcastle University, Newcastle,

12/04-09/05: supported by Royal Society of UK, KC Wong fellowship, Visiting in Heriot-Watt University, Edinburgh

07/00-10/00: Research Associate of Hong Kong Polytechnic University.

06/96-06/00: Associate professor of South China University of Tropical Agriculture.

11/98-07/99: Research Associate of Hong Kong Polytechnic University.

09/97-01/98: Temporary Lecturer of Tsinghua University.

05/94-06/96: Lecturer of South China College of Tropical Crops.

07/91-04/94: Assistant Professor of South China College of Tropical Crops.

Research Interests

Marine Microbial Drug Discovery: marine microbial culture collection, natural products discovery and biosynthesis, drug leads discovery and evaluation of druggability.

Recent Publications

1. Yuan W, et al. **Applied Microbiology and Biotechnology**. 2019. accepted
2. Zhao Y, et al. **Pharmacological Research**. 2019. 146:104294
3. Er-Li Tian, et al. **Tetrahedron Letters**. 2017. 58: 4348–4351
4. Wei Tian et al. **Marine Drugs**. 2017. 15, 229;
5. Manita Kamjam, et al. **Frontiers Microbiology**. 2017 doi: 10.3389/fmicb.2017.00760
6. Chuan-Xi Wang, et al. **J. Nat. Prod.** 2016, 79, 2446–245
7. Ying Han et al. **Molecules** 2016, 21, 970;
8. Chai H, et al. **Sci Rep**. 2016 Jun 7; 6:27181.