Characterization and Heterologous Reconstitution of Taxus Biosynthetic Enzymes Leading to Baccatin III

Bin Jiang1, Lei Gao2, Haijun Wang2, Yaping Sun1, Xiaolin Zhang1, Han Ke2, Shengchao Liu1, Pengchen Ma4, Qinggang Liao1, Yue Wang1, Huan Wang3, Yugeng Liu1, Ran Du1, Torben Rogge4, Wei Li1, Yi Shang7, K. N. Houk4, Xingyao Xiong1, Daoxin Xie5, Sanwen Huang1, Xiaoguang Lei2,6, Jianbin Yan1

1 Agricultural Genomics Institute at Shenzhen, Chinese Academy of Agricultural Sciences; Shenzhen, China. 2 Department of Chemical Biology, College of Chemistry and Molecular Engineering, Peking University; Beijing, China. 3 College of Life Sciences, South China Agricultural University; Guangzhou, China. 4 Department of Chemistry and Biochemistry, University of California, Los Angeles; Los Angeles, CA, USA. 5 Tsinghua-Peking Joint Center for Life Sciences, and School of Life Sciences, Tsinghua University; Beijing, China. 6 Institute for Cancer Research, Shenzhen Bay Laboratory; Shenzhen, China. 7 Yunnan Key Laboratory of Potato Biology, The CAAS-YNNU-YINMORE Joint Academy of Potato Sciences, Yunnan Normal University; Kunming, China.

#Co-first author. *Corresponding author.

Abstract: Paclitaxel is a well-known anticancer compound. Its biosynthesis involves the formation of a highly functionalized diterpenoid core skeleton (baccatin III) and the subsequent assembly of a phenylisoserinoyl side chain. Despite intensive investigation for half a century, the complete biosynthetic pathway of baccatin III remains unknown. Here, we identified a bifunctional cytochrome P450 enzyme (Taxane oxetanase, TOT) that catalyzes an oxidative rearrangement in paclitaxel oxetane formation, representing a previously unknown enzyme mechanism for oxetane ring formation. We created a screening strategy based on the taxusin biosynthesis pathway and uncovered the enzyme responsible for the taxane oxidation of the C9-position (T9aH). Finally, we artificially reconstituted a biosynthetic pathway for the production of baccatin III in tobacco.

Background and Significance

Paclitaxel, derived from the secondary metabolism of Taxus genus plants in Taxaceae, has been clinically used to treat various cancers.

- Important ovarian, breast cancer treatment worldwide.
- Global demands exceed a metric tonne annually.
- Structural core available from European Yew Taxus Baccata allows semi-synthesis, production

Biosynthesis of Paclitaxel:

- taxadiene synthase
- 3 x enzyme functionalization
- benzoyl transferase
- 5 catalytic transformations in one cascade process
- Paclitaxel

Identification of T9aH1 for C9 hydroxylation

Biosynthetic pathway constitution of baccatin III

Acknowledgements:

The National Natural Science Foundation of the Peoples’ Republic of China.
The National Key Research and Development Program of China.
The Beijing Outstanding Young Scientist Program.

Research on bioactive plant natural products:
(1) Biosynthesis of plant natural products.
(2) Identification of new functional plant natural products.

https://www.chem.pku.edu.cn/leigroup/index.htm

w_hj@pku.edu.cn