RESEARCH HIGHLIGHT

Metabolic engineering of the paclitaxel anticancer drug

Audrey Oudin[1], Nicolas Papon[2] and Vincent Courdavault[1]✉

Cell Research (2024) 0:1–2; https://doi.org/10.1038/s41422-024-00950-3

In a recently published *Science* article, Jiang et al. unraveled a couple of missing enzymes achieving key steps in the paclitaxel biosynthetic pathway in yew and reconstituted the production of the main precursor baccatin III in a heterologous host. This advance provides unprecedented perspectives for biotechnological production of this prominent anticancer drug.

As bacteria and fungi, medicinal plants produce a highly diversified series of biocompounds with potent activities for human health. The history of pharmacopoeias reveals that the most powerful natural anticancer drugs we use today in clinical settings have mainly been developed from molecules of plant origin. This is specifically the case of paclitaxel (Taxol®), a natural product synthesized by yew trees (belonging to the *Taxus* genus). Discovered half a century ago and thirty years after its first approval, this microtubule-stabilizing compound is now used worldwide for chemotherapeutic treatments of ovarian, breast, and lung cancers, as well as Kaposi's sarcoma.[1] Like monoclonal antibodies and also several other plant-derived drugs such as lignans from the mayapple podophyllotoxin or monoterpene indole alkaloids from the Madagascar periwinkle, paclitaxel has progressively become an indispensable component of our anticancer arsenal. This is attested by the never-ending rising of the market size of paclitaxel valued at more than 5 billion USD in 2023 and estimated to reach 15 billion USD by 2032.[1] Importantly, as observed for most of the valuable plant natural products, paclitaxel naturally accumulates at a very low rate in planta. Thus, a supply strategy based on the sole extraction from plant material would be rationally way far from sustainability. In addition, paclitaxel has always been recognized in the field of plant specialized metabolism as one of the natural compounds displaying an exceptional molecular structure, making bulk chemical synthesis impossible in a cost-effective manner. In this regard, the supply of this important biopharmaceutical is currently strongly dependent on plant source material (in vitro cell cultures or raw tree biomass) for producing the precursor baccatin III, the latter being finally chemically modified to yield the active product paclitaxel (Fig. 1).[2] As a consequence, the whole industrial process of paclitaxel manufacturing is highly costly due to low production rates. At a time when existing supply procedures for plant anticancer drugs make it no longer possible to satisfy the ever-growing market, it would be of utmost urgency to propel innovations aiming at implementing new, efficient, and sustainable processes for the manufacturing of most of these precious natural products. Alongside the emergence of synthetic biology in the 2000's as initially instilled by the pioneering production of the antimalarial plant bioactive artemisinin by tailored microbes, metabolic engineering has progressively provided unprecedented hopes in developing alternative synthesis strategies for complex biocompounds.[3] In such a perspective, an essential prerequisite is the detailed knowledge of the complete set of biosynthetic enzymes enabling the source organism to produce the valuable natural product. This is mandatory for transferring the complete biosynthetic pathway into a heterologous host that could in turn produce this compound at high titers. Unfortunately, while more than three decades of active research have allowed the identification of most of the biosynthetic enzymes involved in paclitaxel synthesis, our knowledge of the architecture of this biosynthetic route remained incomplete until now and a few enzymes had still to be identified. In an exciting recent development, the Xiaoguang Lei and Jianbin Yan research groups have identified a couple of missing enzymes from the paclitaxel biosynthetic pathway in yew and achieved the production of the main precursor baccatin III in a heterologous expression system.[4]

The paclitaxel biosynthesis can be split in three main stages including (i) the formation of taxadiene-5α-ol from geranylgeranyl pyrophosphate (GGPP), (ii) the conversion of taxadiene-5α-ol to the precursor baccatin III, (iii) and the attachment of a phenylisoserine on baccatin III to yield paclitaxel (Fig. 1). All enzymes involved in stages i and iii were previously characterized. However, some essential enzymatic steps in stage ii, notably those contributing to the formation of an oxetane ring and a C9 hydroxylation, remained hitherto unknown. First, theoretical pathway mining allowed Jiang and colleagues to speculate the involvement of a cytochrome P450 activity in the generation of the oxetane ring on the tricyclic intermediate. On this basis, they developed a powerful screening strategy allowing to simultaneously test the activity of several dozens of candidate genes combining genome mining, phylogenetic inference, and transient gene expression in benth tobacco (*Nicotiana benthamiana*). This led to the identification of the bifunctional yew taxane oxetanase 1 (TOT1) as responsible for this crucial step. In a second series of experiments aiming at characterizing the missing enzyme performing C9 hydroxylation of the tricyclic backbone, Jiang and colleagues proceeded through a guilty by association strategy reasoning that the corresponding gene could share a similar expression profile with the 7 previously identified biosynthetic enzyme genes involved in baccatin III synthesis. Thus, by browsing yew multi-omics resources, they selected a set of 17 candidate genes for the missing activity. Further transient gene expression experiments in benth tobacco led to the discovery of taxane-9α-hydroxylase 1 (T9αH1) performing the essential C9 hydroxylation

[1]Biomolécules et Biotechnologies Végétales, BBV, EA2106, Université de Tours, Tours, France. [2]Univ Angers, Univ Brest, IRF, SFR ICAT, F-49000 Angers, France.
✉email: vincent.courdavault@univ-tours.fr

Published online: 14 March 2024

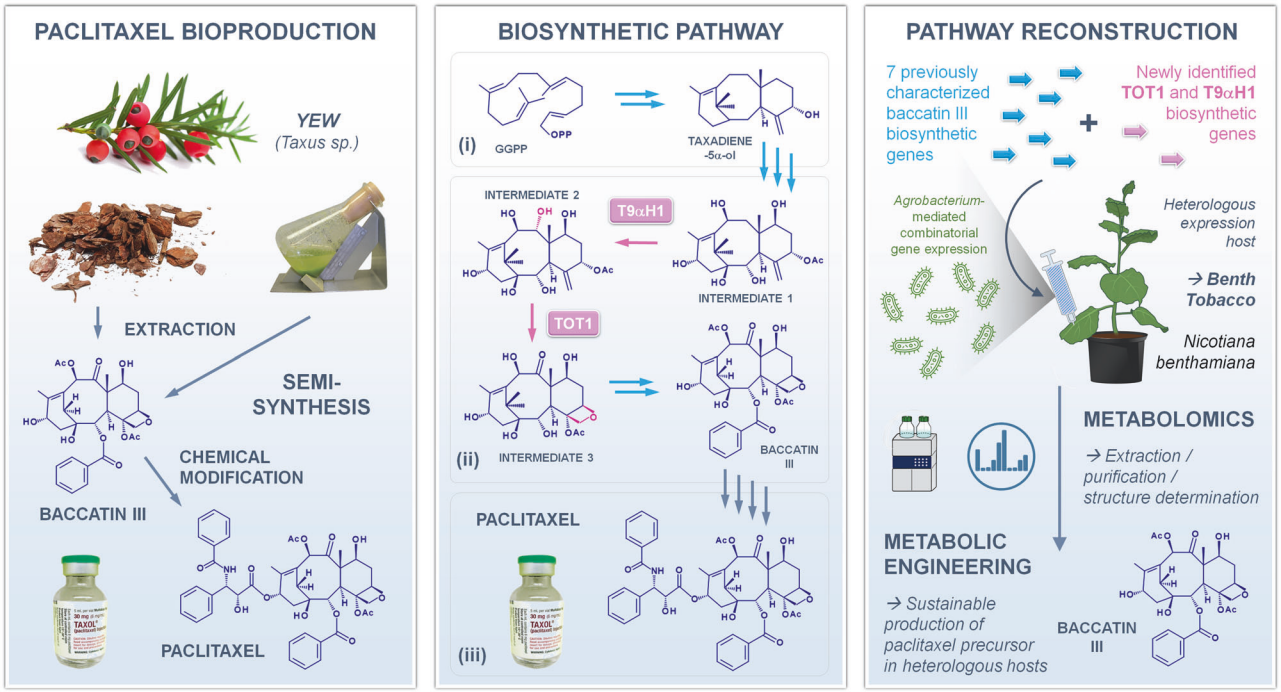

Fig. 1 Identification of missing yew enzymes and reconstruction of the baccatin III biosynthetic pathway in a heterologous host. The current semisynthetic supply process for paclitaxel is based on extraction of the precursor baccatin III from yew bark or cell cultures. Subsequent chemical modifications on baccatin III yield paclitaxel. The paclitaxel biosynthetic pathway in yew is divided into three main stages (i, ii, iii). Jiang and colleagues report the characterization of a couple of new enzymes involved in stage ii of the pathway namely the taxane-9α-hydroxylase 1 (T9αH1) that hydroxylates the tricyclic intermediate 1 at position C9 and the bifunctional yew TOT1 that generates the oxetane ring on intermediate 2.[4] Finally, they successfully achieved the baccatin III synthesis in benth tobacco by co-expressing the 7 previously identified biosynthetic genes involved in stages i and ii along with TOT1 and T9αH1.

in baccatin III formation (Fig. 1). Finally, by transferring and co-expressing the 7 previously identified genes encoding the biosynthetic enzymes involved in stages i and ii along with TOT1 and T9αH1, Jiang and colleagues successfully reconstructed the baccatin III synthesis pathway in benth tobacco (Fig. 1).

Taken together, all the data collected by Jiang and colleagues definitely constitute a breakthrough in the field of metabolic engineering of natural products. Beyond the identification of some missing and recalcitrant enzymes of paclitaxel biosynthesis, they provide a formidable proof of concept for the heterologous production of the precursor baccatin III. While their preliminary attempts have produced only trace amounts of this intermediate (50 ng per g dry weight), there is considerable enthusiasm that further improvements could be achieved soon to reach industrial titers. In line, the report by the Lei and Yan research groups adds to other recent plant metabolic engineering studies that highlight the terrific potential of combining high-quality genome resources in medicinal plants, phylogenomic analysis, massive transcriptomic and metabolomic data, with new powerful combinatorial transient expression systems in various eukaryotic chassis. In recent years, these major technological advances have indeed accelerated both elucidation of extremely complex plant specialized metabolic pathways and facilitated their reconstitution in heterologous host.[5–9] Finaly, this excellent report teaches us once again how the substrate promiscuity of some enzymes — inherent to the evolution and diversification of plant specialized metabolic pathways — could be also exploited for the production of new-to-nature metabolites with interesting/improved activities.[10] In conclusion, plant metabolic engineering is now definitively ripe for future innovations in both sustainable supply processes and therapeutic development.[11]

REFERENCES

1. Sofias, A. M. et al. *Adv. Drug Deliv. Rev.* **122**, 20–30 (2017).
2. Perez-Matas, E. et al. *Front. Plant Sci.* **13**, 942433 (2022).
3. Paddon, C. J. et al. *Nature* **496**, 528–532 (2013).
4. Jiang, B. et al. *Science* **383**, 622–629 (2024).
5. Zhang, J. et al. *Nature* **609**, 341–347 (2022).
6. Reed, J. et al. *Science* **379**, 1252–1264 (2023).
7. Nett, R. S. et al. *Nature* **624**, 182–191 (2023).
8. Zhao, Y. et al. *J. Am. Chem. Soc.* **146**, 801–810 (2024).
9. Zhang, Y. et al. *Mol. Plant* **16**, 1951–1961 (2023).
10. Bradley, S. A. et al. *Nat. Chem. Biol.* **19**, 1551–1560 (2023).
11. Biggs, B. W. et al. *Science* **374**, 1563–1565 (2021).

ADDITIONAL INFORMATION

Correspondence and requests for materials should be addressed to Vincent Courdavault.