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学 位 論 文 内 容 の 要 旨

博士の専攻分野の名称　　博士（理学）　　氏名　Yaping Liu

学 位 論 文 題 名

Biosynthetic Studies of Fungal Terpenoids and Meroterpenoids Having Antiinsectant and Antitumor Activity
（抗虫活性および抗腫瘍活性を有する真菌テルペノイドおよびメロテルペノイドの生合成研究）

Fungi are a prolific source of bioactive natural products (NPs). These NPs play an important role in health and agricultural applications. Representative example is the antibiotic penicillin and the cholesterol-lowering agent lovastatin. The wide range of biological activities of NPs is highly dependent on their characteristic structural diversity. The structural diversity of fungal NPs is constructed by oxidative modifications. These oxidative modification processes can be catalyzed by oxidation enzymes. One of the key enzymes is the cytochrome P450s, which are a large superfamily of hemoproteins. They can catalyze several reactions, such as a hydroxylation and desaturation, in a regio- and stereoselective manner. Another important oxidation enzyme is the flavin-dependent enzymes, which can catalyze substrate oxidations involving oxidative cyclization reactions. Taken together, the functional analysis of these oxidation enzymes is essential to elucidate the structural diversification mechanism of fungal NPs. In this thesis, I focused on the oxidative modification enzymes for the synthesis of structurally diverse NPs, including fungal terpenoids and meroterpenoids. In chapter 2, I achieved the synthesis of unnatural indole diterpenes, and in chapter 3, I achieved the identification of key enzymes involved in the A ring modification process of hirsutane.

In chapter 2, I performed biosynthetic studies of the indole diterpenes nodulisporic acid (NA) and shearinine (SN). Using heterologous expression experiments, I characterized two key enzymes, a flavoprotein oxidase NodO and a cytochrome P450 NodJ, responsible for the biosynthesis of structurally diverse noduliporic acids (**Figure 1A**). NodO catalyzes the formation of characteristic bicyclic ring found on the indole ring and NodJ mediates the side chian modification processs. I also examined the enzymatic synthesis of NA- and SN-type bicyclic rings through in vitro enzymatic experiments. Specifically, I revealed that NodO exhibits strict substrate specificity toward pentacyclic IDTs to afford NA-type bicyclic ring, while JanO can accommodate penta/hexacyclic IDTs to synthesize the SN bicyclic ring. The broad substrate tolerance of JanO provides opportunities for the synthesis of unnatural IDTs (**Figure 1B**).

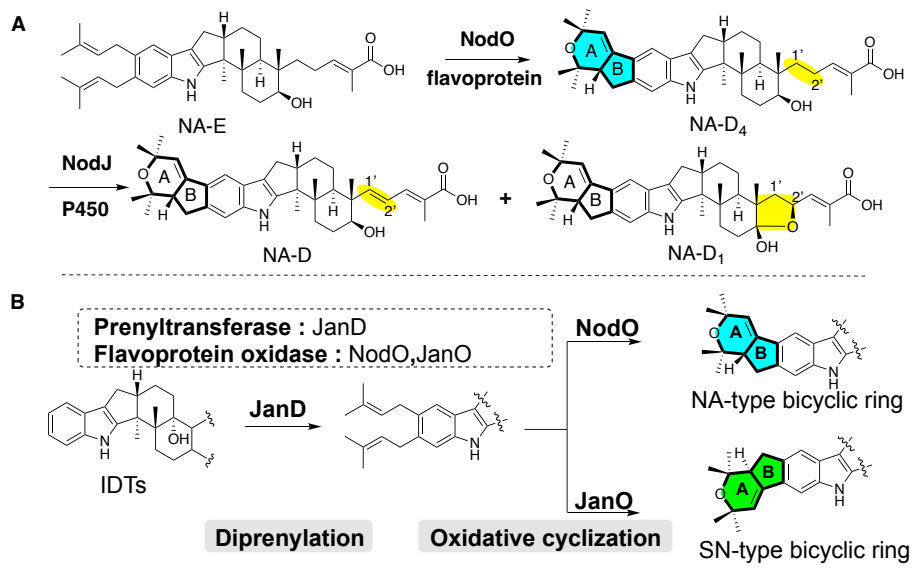

Figure 1. (A) Oxidative modification by NodO and NodJ. (B) Oxidative cyclization catalyzed by NodO and JanO.

In chapter 3, I performed the biosynthetic study of the *Basidiomycota* sesquiterpene hirsutanes containing more than 80 derivatives. They commonly share the 5-5-5 tricyclic ring system, however the diverse oxidative modification patterns on the A-, B-, and C-rings contribute to their structural diversity (**Figure 2A**). To elucidate the structural diversification mechanism, I conducted heterologous expression experiments using *A. oryzae* and identified the key cytochrome P450s, HirC and HirF, involved in the A ring biosynthesis of hirsutane. HirC catalyzes the stepwise hydroxylation at the C6 and C4 positions to afford mono- and di- hydroxylated products, **3-7** and **3-8**. By contrast, HirF participates in the stepwise hydroxylation reactions at the C4 and C7 positions to give **3-9** and **3-10**. The successful functional analysis of HirC and HirF enable to hypothesize the oxidative modification pathways on the A ring for the synthesis of structurally diverse hirusutanes. Specifically, compounds, **3-8** and **3-9**, possesse a common partial structure of group III hirsutanes. Subsequent C4 oxidation followed by dehydration gives an α,β-unsaturated moiety commonly found in group II hirusutanes. Finally, epoxidation affords group I hirusutanes (**Figure 2B**). The proposed biosynthetic pathway provides opportunities to elucidate the complete biosynthetic pathway of hirsutanes.

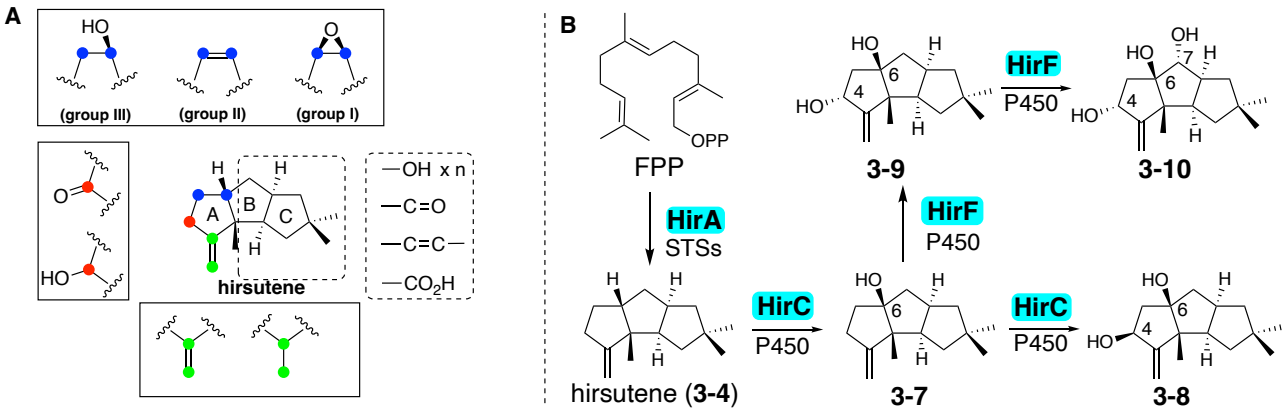

Figure 2. Oxidative modifications on the A ring of the hirsutanes.