学　位　論　文　内　容　の　要　旨

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学　位　論　文　題　名

Reconstitution of biosynthetic gene clusters for bioactive fungal metabolites

Fungi grown in different environments produce various secondary metabolites (SM) during their life time. Most of these fungal SMs exhibit bioactivities, such as penicillin isolated from *Penicillium* species as the antibiotic drug, and aflatoxins isolated from *Aspergillus flavus* as carcinogenic mycotoxins. Studies on biosynthesis of these fungal secondary metabolites not only help to produce the useful compounds but also to have a deeper knowledge of harmful toxics to avoid their adverse effects. This thesis is focusing on reconstitution of biosynthetic gene clusters for bioactive fungal metabolites, and is composed of 5 chapters.

Chapter 1 gave an introduction of *Aspergillus oryzae* heterologous expression which has been developed to be an efficient and reliable system for heterologous expression of fungal biosynthetic enzymes. Using this system, natural products involving multiple biosynthetic genes such as Aflatrem (7 genes) have been totally biosynthesized and biosynthetic pathway of complex natural products such as penitrem A (17 genes) have been elucidated.

In **Chapter 2**, genome mining of bifunctional di/sesterterpene synthase was conducted in *A. orzyae*, Phylogenetics analysis of bifunctional terpene synthases from fungal genomes were performed and four candidates from different clades were chosen. The candidate genes were introduced into *A. oryzae* among which *NfSS* transformant produced a novel sesterterpene named sesterfisherol.

The cyclization mechanism of NfSS was elucidated from *in vivo* and *in vitro* labeling experiments and exhibited its uniqueness through a series of hydride shifts. On the basis of this mechanism, a unified biogenesis for group A sesterterpenes from bicyclic (5-15), tricyclic (5-12-5) and tetracyclic (5-6-8-5) cation intermediates was proposed. Especially, the initial cyclization mode of each synthase may be reflected by phylogenetic clades it belongs to. Although not yet proved, this promising hypothesis suggested that phylogenetic analysis will be the roadmap to guide future genome mining of novel

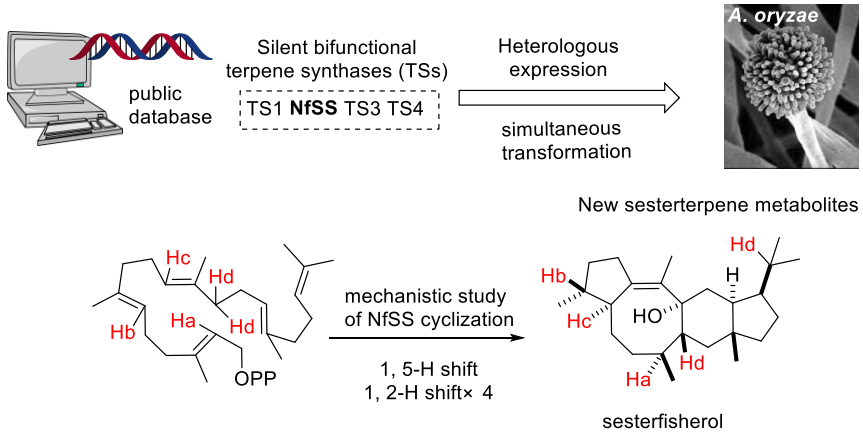

Figure 1 Genome mining for bifunctional terpene synthases

di/sesterterpenes.

In **Chapter 3**, the author focused on the biosynthetic pathway of ustiloxin B, whose biosynthetic gene cluster (BGC) was the firstly identified RiPPs BGC from filamentous fungi. At first, based on the structural elucidation of the metabolites accumulated in a series of gene deletion mutants of *A. flavus*. The biosynthetic pathway of ustiloxin B was proposed. Heterologous expression of *ustAQYaYb* revealed that these four genes were involved in the biosynthesis of the first cyclic intermediates, *N*-desmethylustiloxin F. Especially, UstYa and UstYb, both of which contained Duf3328, were proposed as novel oxidative enzymes responsible for the formation of ether linked macrocycle. The enzymes involved in the late stage using recombinant enzymes were also characterized. The FAD-dependent UstF2 catalyzed two rounds of hydroxylation on amino group followed by decarboxylative dehydration. The PLP-dependent UstD catalyzed unprecedented C-C bond formation through a decarboxylative condensation. Based on the above results, the biosynthetic pathway of ustiloxin B was characterized in detail.

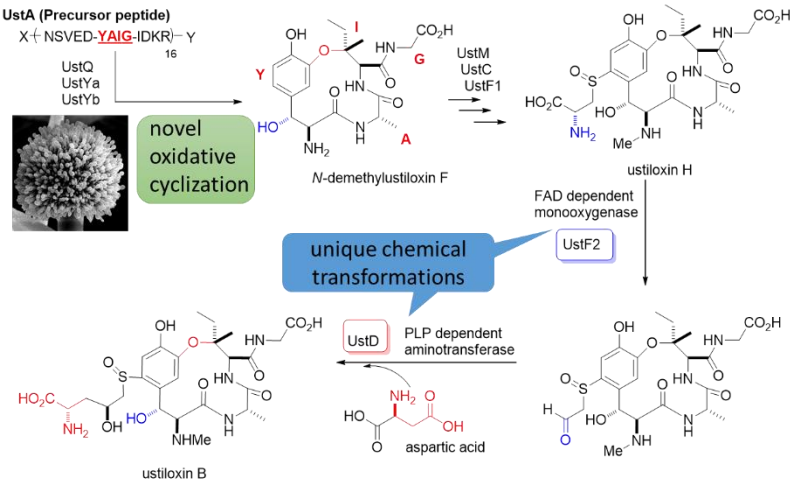

Figure 2. Biosynthetic pathway of ustiloxin B

In **Chapter 4**, asperipin-2a, which was recently discovered by bioinformatics study, was chosen as the subject for biosynthetic study on fungal RiPPs. Four genes *aprAYRT* were introduced into *A. oryzae*. Resultant AO-*aprAYRT* produced asperipin-2a. confirming that these four genes were actually involved in the biosynthesis. Notably, AprY likely catalyzed two oxidative cyclizations to form the two ether bonds of asperipin-2a. This result also indicated that ustY homologues, which contained Duf3328 and were often found in fungal RiPPs gene clusters, would be universal enzymes that were responsible for oxidative cyclization. In addition the absolute configuration of asperipin-2a was also determined, taking advantage of the high production achieved by the *A. oryzae* heterologous expression.

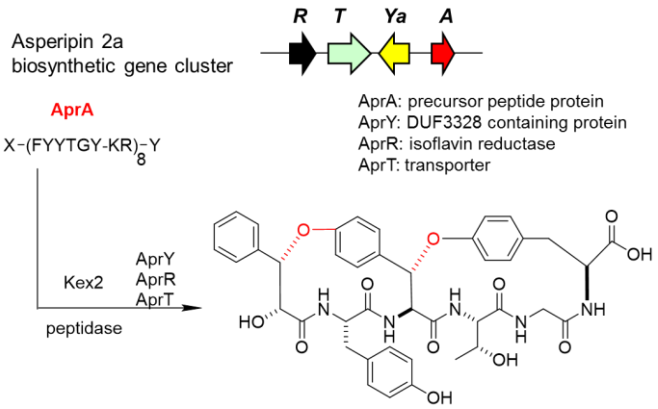

Figure 3. Production of asperipin-2a in *A. oryzae*

Chapter 5 gave a summary. This thesis has shown that *A. oryzae* is a powerful heterologous expression host to discovery novel natural products and to study the novel biosynthetic enzymes. The NfSS and UstY homologues were established as "probes" for the future genome mining for novel di/sesterterpenes and fungal RiPPS.